BIOLOGICAL PROCESSES AND SOIL FERTILITY

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Biological Processes and Soil Fertility

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Preface

The success of shifting cultivation systems developed by subsistence farmers testifies to the resilience of the "natural" soil-plant ecosystems to recover from the offtake of nutrients in crops and loss of soil structure. By contrast, the development of intensive cropping systems requires large inputs especially of nitrogen, together with phosphorus, sulphur and other essential elements in order to maintain the nutrient levels needed for abundant crop yields.

As Dr. Cooke ably pointed out in his introductory lecture, the discoveries and experiments of the 19th century encouraged farmers in temperate zones to rely greatly on chemical fertilizers supplements. However, the work of Charles Darwin on soil mixing by earthworms and the discovery by Hellriegel and Wilfarth in 1886 that the nodules on legume roots contain colonies of symbiotic bacteria able to "capture" atmospheric nitrogen molecules to the benefit of the host plant heralded a growing realization of the importance of soil biota in fertility studies.

Biological fixation of nitrogen has been the theme of many meetings and publications hitherto but at this Conference, convened on the delightful campus of Reading University, attention was mainly focussed on other biological processes in soil fertility. These Proceedings record the dominant themes and include six keynote addresses delivered at plenary sessions and seven introductory lectures to paper reading sessions by invited individuals plus 22 of the proferred papers, in six sections as tabled in the contents list.

We are sorry that the space available under contract with the Publisher did not allow for more papers to be published: some of those not included were in fact reserved by authors for publication elsewhere. The abstracts of all papers delivered orally or displayed by poster are contained in the Conference Transactions supplied to each participant.

At the concluding session Dr. Greenland gave a masterly review of the Conference achievements in the light of global developments and demands, pointing to the tremendous advances in quantitative studies of the various sectors of the nitrogen cycle through the use of ^{15}N and ^{13}N isotope techniques; to greatly improved understanding of the organic phosphorus accumulations in soils; to the dearth of reports on sulphur mentioned by Dr. Cooke; and to the tremendous interest in the living biomass components of soil organic matter made possible

by new techniques, particularly chloroform fumigation and ^{14}C labelling.

Many will endorse his view that experimental techniques now need to be adapted further to the study of crop management considerations and to modelling the distribution of specific soil organisms or groups of them according to biological conditions in the field: crop pathogens had been hardly mentioned. Since rice is the staple food of half the world's population, perhaps the next such conference should concentrate on soil biotechnology and be held at an appropriate tropical location.

The Organizing Committee wishes to record grateful thanks to the McRobert Trust for substantial help towards the cost of publishing these Proceedings, and to all who helped in any way to make the Conference such a notable success.

Aberdeen

J. Tinsley and
J. F. Darbyshire

The application of soil science to increasing soil productivity

An introduction to the Conference

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Summary The Conference will discuss biological processes that have an essential role in maintaining and increasing soil productivity. Public interest in biological aspects of agriculture, and in avoiding pollution of the environment, supports our efforts to manage agricultural systems more efficiently. Improved systems that maximise the use of natural resources, and of biological processes that increase soil fertility, are needed to produce more food for the rapidly expanding populations in developing countries.

The history of research in soil science in Britain is reviewed. Great increases in agricultural productivity, achieved in the last 50 years in this country, owe much to the application of this research. Soil and water management, and crop nutrition, have been much improved; biological research has shown the importance of soil organisms, some of which aid plant growth while others cause damage.

Further progress requires the integration of biological, physical, chemical and agronomic work on the ecology of crop production. More research is needed to breed plants which develop beneficial associations with soil microorganisms, on the effects of the soil population on physical properties of soil, and on the cycles of nutrient elements in which microorganisms are involved. All research that is planned to develop the ability to manage soil fertility efficiently and reliably requires the full collaboration of workers of different disciplines. From their joint work they will develop new technologies which, when applied, will give increased output with the maximum efficiency of all inputs, and with no losses to the environment.

Introduction

I must first welcome you to this Conference of Commissions III (Soil Biology) and IV (Soil Fertility and Plant Nutrition) of the ISSS which is being held here in Reading, the principal city of the Royal County of Berkshire. The University that is our host has a tradition of work on soils that goes back to the beginning of this century. I believe that joint meetings of this kind between soil scientists concerned with different disciplines are very important because progress will be made by the integration of our science resulting from discussions on common ground and common problems.

Modern agriculture is a science-based industry which operates in an ecological environment that has been created by farmers, and which may seem far from the natural association of plants and soil that it replaced. But the essential components of the systems – living organisms

and inert structures – are the same. The study of the interactions between the organisms themselves, and between the organisms and their surroundings, – which we call ecology – is the background of our work to understand the components of soil fertility better and to develop the ability to manage the system to our advantage. Scientific management of cropping systems requires the integration of our knowledge of the components of soils and of the biological, chemical and physical processes that occur in them; I see this as our purpose here in Reading.

The programme is well designed to further the objectives of the two Commissions. Professor Paul prepared a Chairman's report on the proceedings of Commission III at the 12th Congress in New Delhi last year and every topic he mentioned is of vital importance to the concern of Commission IV with soil fertility and crop nutrition. Similarly many of the recommendations made by Hera on the proceedings of Commission IV have implications for Commission III, notably in fertilizer efficiency, cropping systems, the use of indigenous sources of nutrients, the biological fixation of nitrogen, and for soil-plant-environment research to be considered as a whole.

The role of this Conference

The presence is a very appropriate time for this meeting for a number of reasons; I will mention three:

1. The first is the need to review present work and from these reviews to develop concepts which will lead to new technologies for application to practical farming. These applications will raise the productivity of agriculture and also the efficiency of the inputs employed. I shall return to this objective in more detail later.

2. The second reason is that many are now questioning the basis of modern intensive agriculture. The energy that is needed to cultivate the land and to make fertilizers is criticised (unjustly in my view); the inputs of inorganic fertilizers are criticised as is the extensive use of agrochemicals to control weeds, pests and diseases of crops. (The effects of agrochemicals on ecological processes in soils will be discussed later in our meeting.) The critics are usually supporters of systems of 'Biological Husbandry'. In recent years the defenders of intensive farming, and the supporters of organic farming have had rational discussions that seemed impossible years ago when uncompromising attitudes were expressed by Sir Albert Howard and his disciples in the 1930s. An example was the useful Conference held at Wye College in 1980[16]. If polarised attitudes can be avoided the two 'sides' will learn much from each other. We all support the emphasis

that organic farmers give to maximising the biological fixation of nitrogen, and the rational use of all wastes so that nutrients are efficiently recycled. Their emphasis on the biological control of pests is important in diminishing the risk of resistance to pesticides developing. In turn the exponents of organic farming recognise that larger yields can only be achieved by increasing the supplies of nutrients and that fertilizers have an important role in enlarging nutrient cycles. Consequently they seek to provide extra nutrients from natural minerals found in the earth. Any research that is done to support organic farming will be of general benefit by improving understanding of the whole biological system in the soil and by evaluating the effects of chemical inputs on this system.

Some urban dwellers far removed from agriculture are concerned about the effects of the farming that provides them with adequate food that is cheap and of high quality, on the purity of the air and of the natural waters used for public supply or amenity. Although the concerns of these critics for the effects of pollution from agriculture on their health and welfare may not be fully justified, we must note that our objectives in improving agricultural efficiency by preventing losses of plant nutrients from farming systems are the same as those of people who deplore any taint from agriculture in their water or environment. For these reasons environmental research and agricultural research should be closely coordinated and this will benefit both farmers and consumers.

3. My third reason for approving of the timing of this Conference is the present world food situation which must be assessed against the rapidly increasing population – expected to rise by nearly 50% to over 6 billion people by the end of the century. In the developing countries many people now suffer from ill-health caused by inadequate diets. To rectify the present situation, and to provide for the future, food production in these countries must be greatly increased; to be safe we should plan to have the ability to double the present level of yields in developing countries in the next 20 years. The obstacles to a speedy solution of this most difficult problem are the shortages of resources to adopt the intensive systems employing large inputs of fertilizers and other chemicals that have raised yields so markedly in recent years in developed countries. I think we will only overcome this difficulty when governments give the highest priority to agriculture and to the development of rural resources and facilities. In particular they must provide the support for scientists and agronomists to join in research to develop scientifically-based farming systems for the tropics and sub-tropics that suit local soils and climates and also the

abilities, financial resources and equipment that the farmers may have, and which are compatible with national objectives.

Developing improved agricultural systems

The present systems used in developed countries resulted from a slow process of improvement which proceeded sporadically for a period of 200 years or more, but which has become much more rapid and consistent in the last half century. This long time-scale did not matter because populations did not grow quickly. The present situation in many developing countries is much more urgent and cannot be left to the traditional processes of agricultural improvement by trial and error. The future of agriculture in the developing countries, and indeed of world agriculture, must rest on higher output achieved by *maximum intellectual input,* but with the *costly material inputs minimised* by securing maximum efficiency in their use through research and its application.

Although the emphasis here is on work for the developing countries, I believe that the outcome of much of the research needed will have implications and benefits for all of world agriculture, including the intensive systems now common in temperate countries. Soil scientists do recognise the need to consider systems as a whole and the chemical and/or physical aspects cannot be separated from the biological aspects. The ecology of agriculture describes the changes in the interactions between the living components and the inert components and structures which comprise the rest of the system. The integration and synthesis of much information on processes occurring in the soil now requires mathematical modeling and computer simulation. Much progress has been made in studying processes such as solute movement[8]. But a limit to the usefulness of such work is set by the interactions between the living and the non-living components whcih makes us realise that all components must be included in the 'final' model, however difficult that may be. I am pleased that we are to have a lecture on this subject from Dr van Veen.

Progress in soil science in Britain

Soil science began in Britain in the early years of the last century. Progress may conveniently be described for three periods – to 1875, from 1875 to 1925, and then through the last half century. The account is not complete; I have only outlined some major achievements as a basis for discussions on their application to improved soil management. Fully documented accounts are given by E J Russell[12] and by E W

Russell[13]; early work has been well discussed by Tinsley[17]. For a much wider presentation I commend the Bicentennial Review of the Soil Science Society for America[15].

The foundation – to 1875

Humphrey Davy was the first British chemist to draw together the knowledge of plant nutrition, as then understood, in his lectures and publications. He prescribed the first physical and chemical methods for soil analysis. Although Liebig's work was not done in Britain his famous presentation to the British Association and his writings stimulated much discussion and research. Otherwise it was a dull period until the middle of the century when Daubeney at Oxford first distinguished between 'available' and 'unavailable' nutrients in soil and Way established the principles of base exchange.

Most important in this period was the work of Lawes and Gilbert at Rothamsted. They set out their famous field experiments which established for important British crops the practical basis of nutrition by the use of manures and fertilizers, and laid the foundations of the fertilizer industry. These experiments were to have a vital role by providing working materials for soil science that has extended right up to the present and tribute must be paid to those who have had the wisdom and vision to maintain them in faith that their usefulness would continue. In their pot experiments Lawes and Gilbert disproved Liebig's theory that plants obtained much of their nitrogen as ammonia from the air; but the rigorous chemical discipline which Gilbert imposed on the experiments prevented the legumes grown in sterile conditions from exhibiting their capacity to fix nitrogen from the air. They also measured the water requirements of crops, an initiative that was unfortunately not taken up again for nearly 100 years.

The growth period 1875–1925

By this time the science of microbiology had developed. Warington joined Rothamsted in 1876 and he was the first soil microbiologist there; he showed the role of micro-organisms in converting ammonia to nitrite, and then nitrite to nitrate. However he failed to identify the actual nitrifying organisms (this was achieved by Winogradsky). The proof that legumes obtained nitrogen by bacterial fixation owed nothing to British workers, though it was confirmed by Gilbert at Rothamsted. Unfortunately Warington was driven from Rothamsted by the self-centred Gilbert; he went to Oxford and wrote the first British book on soil physics.

A D Hall became Director of Rothamsted in 1902 and he injected

new life into the old Station, establishing work on botany, microbiology and soil physics. Perhaps the most significant work in this present context was that by E J Russell and H B Hutchinson on the partial sterilisation and related studies in which they were the first to study predator-prey relationships in soil.

Available nutrients in soils This was an important field of work through the period. In developing his 1% citric acid solution Dyer[5] rejected the stronger acid extractants used by earlier workers; his method gave useful service in Britain into the 1940s. Much work was done later to compare solvents for extracting soils and to calibrate the data with the responses of crops to fertilizers. The long-term experiments at Rothamsted were invaluable for this research; by this time they had been supplemented by other long-term experiments and I shall describe the whole group as the 'Classical Experiments'; those at Woburn (Bedfordshire) started in 1876, those at Cockle Park (Northumberland) in 1897 and the Saxmundham (Suffolk) experiments began in 1899. Although these investigations led advisers to select analytical methods that suited their crops and soils, there was little advance in the basic understanding of the processes involved.

Crop nutrition Vast numbers of empirical field experiments were made by local workers to give a practical basis for advising on the fertilising of arable crops and grass. In addition, workers at Long Ashton and East Malling Research Stations established the principles of the nutrition of fruit trees; T Wallace's proof of the importance of potassium was an important feature of the Long Ashton work. The nutrition of glasshouse crops was investigated at Cheshunt (Hertfordshire). Rothamsted workers investigated the role of micronutrients, notably boron.

Soil survey The surveys made by A D Hall and E J Russell in south-east England at the end of last century are well known. Other surveys were made in East Anglia by Cambridge University, in southern England by Luxmore at Reading and by other local workers at Research Stations and Colleges. The early surveys were largely based on the local surface geology, but the workers also developed ideas on the relationships between soil type, land capability and crop responses to fertilizers.

The fruitful period, 1925 to the present

The remarkable feature of the early years of this period was the large expansion in our understanding of soil science, that was achieved

by relatively few workers. All this excellent work was recorded in successive editions of E J Russell's classical text[13] so I have not given detailed references in this paper.

Crop nutrition Experimental work on the role of fertilizers and manures received a considerable impetus from the application of R A Fisher's work on the design of experiments which provided means of measuring the effects of nutrients, their interactions when applied in factorial experiments replaced the simple designs, often unreplicated, used in earlier years. E M Crowther was notable for initiating series of these experiments on selected crops in this and other countries. These experiments were used to calibrate soil analyses against crop responses to fertilizers. The Classical Experiments were also supplemented by other series of long-term experiments, such as those on organic manuring and the ley-fertility experiments done at Rothamsted and Woburn and on the Experimental Husbandry Farms of the National Agricultural Advisory Service (NAAS) established in the 1940s. These latter experiments provided important data on nutrient cycles in complex farming systems.

Considerable advances in basic understanding resulted from R K Schofield's work at Rothamsted on the application of thermodynamic principles to soil/nutrient relationships; from this the concepts of intensity and capacity of nutrient supply were developed. The use of radio-isotopes and of ion-exchange resins made further advances possible. Calibration of new methods of soil examination continued by using soils from the Classical Experiments as well as soils from the large series of modern experiments made on commercial farms. Important events in the 1960s were conferences initiated by NAAS to review the evidence and select soil analysis methods most suited to conditions in England and Wales. The methods selected, and the modes of interpreting the analyses that were decided, together with the results of the large numbers of field experiments since 1930, have given a very sound basis for advising farmers on the use of fertilizers, manures and lime. The use of soil analysis was supplemented by the advances in the application of plant analysis resulting from work at several Stations, but notably at Long Ashton. Work at Cheshunt, which was continued at the Glasshouse Crops Research Institute when this was formed in 1956, led to liquid feeding of glasshouse crops and subsequent widespread adoption of the Nutrient Film Technique (NFT).

Micronutrient deficiencies Many examples of these deficiences

occurred with the intensive arable cropping necessary during the 1939–45 war where large areas of land previously in permanent grass were ploughed. Important work was done at Long Ashton in the Unit of Plant Nutrition established by the Agricultural Research Council (ARC). Highly-developed pot culture methods were used to establish deficiency symptoms which were illustrated in an atlas prepared by Wallace[18]. Basic work was done on the role of micronutrients, for example by E J Hewitt on molybdenum. Much practical work on the diagnosis of trace element deficiencies, and their correction, was done by the advisory services. In Scotland, workers at the Macaulay Institute, notably R L Mitchell, achieved an international reputation for developing instrumental methods for measuring in soils and in plants the concentrations of trace elements that are important in plant and animal nutrition.

Soil survey Contributions to the philosophy and methodology of soil classification and surveying were made by G W Robinson (North Wales) and by W G Ogg (Scotland). Robinson was much involved in the early work of the ISSS in standardising particle size analysis. It was accepted that soil classification should have a genetic basis developed from profile examinations. Ogg was responsible for the formation of the Macaulay Institute for Soil Research at Aberdeen in 1930 and he quickly established the Soil Survey for Scotland as a Department of the Institute. The Soil Survey for England and Wales was established under Robinson at Bangor in 1939; its headquarters moved to Rothamsted under A W Muir in 1946 but Robinson continued to give guidance as Chairman of the Advisory Board.

The Scottish Survey has provided maps of nearly all the lowland suited to modern farming and justifying detailed mapping. Mapping on a detailed scale of the much larger lowland area of England and Wales has proceeded well but the task will not be completed until the 1990s; the whole of Great Britain will shortly have a soil map on the 1:250,000 scale. The classification used in England and Wales is described by Avery[1].

The application of soil surveys was slow in the early years, perhaps because the pedologists involved were apt to study the soil as a natural object and did not consider so much its relation to agriculture. Good progress has been made in the last 20 years with assessing the agricultural value of the soils mapped and the preparation of Land Use Capability maps is now a routine activity. An important development now being planned is the preparation of computer-based information services which will supply to all who need it the information available

for a particular site on classification and capability, and the physical and chemical properties of the soil. Such information is essential to modern management systems; these 'data-bases' will become vital tools in the future control of soil productivity wherever modern farming is established.

Soil physics The physical properties of soil were much studied by B A Keen and his colleagues at Rothamsted in the early years of this period and the factors governing soil porosity were established. Much was done on soil-water relationships. R K Schofield established the pF concept. The physics of evaporation of water by crops was studied by H L Penman who established a firm basis for calculating the water used which led to a scientific base for the application of irrigation. The Unit of Soil Physics was established by the ARC at Cambridge under E C Childs to consolidate earlier work at that University on the drainage of clay soils. It did fine work on the theory and practice of water movement in soils leading to a scientific basis for draining soils which has been widely applied here and overseas.

The need for much cultivating of soils had been questioned by Davy. B A Keen and E W Russell initiated experiments in the 1930s which showed that anything beyond the stirring needed to control weeds, or to prepare a seedbed, was often unnecessary and sometimes harmful. These results have, of course, led to modern systems of minimum cultivations and to 'no-tillage' cropping.

Soil biology Important studies on the interactions between soil micro-organisms were made at Rothamsted. Work on the fixation of nitrogen by Rhizobium was done by H G Thornton and continued by P S Nutman. Much was also done on the microbial decomposition of organic materials, including crop residues, in soil. Work on these topics has been effectively continued and the programme has expanded to include studies of other nitrogen-fixing symbioses, the associations of fungi with plant roots that assist nutrient uptake, and the effects of agrochemicals on microbial life in the soil. Recent work on the selection of strains of Rhizobium at the John Innes Institute now offers the possibility of constructing improved strains by combining the desirable features from different parental strains in a hybrid. Zoologists at Rothamsted and elsewhere have studied the larger animals (notably earthworms) that occur in soil and have a significant role in the organic matter cycle.

The advances made by the early workers, and the popular interest aroused, can be judged by the response to the book 'Microbes by the

Million' written by chemist turned microbiologist – Nicol[7]. The ARC also saw the possibility of advances being made through more detailed studies of the biological processes in the soil. They established their first Unit – of 'Soil Enzyme Chemistry' at Rothamsted in 1941 under J H Quastel. The name was later changed to 'Unit of Soil Metabolism'; the Unit moved to South Wales in 1945 and was disbanded in 1947. Techniques for treating a column of soil in the laboratory as though it was an intact biological entity were developed to follow microbiological transformations of nitrogen, carbon and manganese in soil, and to study the formation of substances which stabilise structure. It is unfortunate that the initiative created by the Unit was lost; perhaps it was because collaboration with other disciplines was lacking at that time; more probably that Quastel left for Canada.

Recent work on the biological effects of waterlogging soils has been done at the Letcombe Laboratory. We now recognise that comprehensive work on soil fertility must include biological and biochemical examinations; we are fortunate that Professor Waid will speak to us on this subject.

The composition and properties of soils Warington originally came to Rothamsted in 1876 to study changes in the carbon and nitrogen in soils and work on this topic has continued to this day. In the 1920/30 period H J Page studied the fractionation of soil organic matter and H L Richardson studied the nitrogen cycles in different land-use systems. Work on organic matter was continued in the 1950s by J M Bremner who took advantage of modern methods in organic chemistry to pursue the fractionation of both the nitrogen and the carbon. Later D S Jenkinson studied the carbon and nitrogen cycles in natural conditions and in cropped soils; he has applied ^{15}N to the study of the nitrogen cycle, with notable success in accounting for nearly all of the N applied as fertilizer.

Early work on the mineral fraction, and particularly the properties and constitutions of clays, and the ionic reactions between soils and electrolytes, was done at Rothamsted and at Leeds University. Work on clay minerals became an early feature of the programme at the Macaulay Institute, which acquired a high reputation for developing instruments for the analysis of minerals in soils and for measuring their properties. Later study of soil clays at Rothamsted was done in the Pedology Department headed by A W Muir and, subsequently, by C J Bloomfield. Much work was also done in the same Department on the nature of soil structure and the porosity of soils. All of this work on the mineral fraction has been of great help to the pedologists

concerned with the properties and classification of soils in the field. Its application to practice has been through the guidance given to those concerned with soil survey and classification and to others concerned with the use of fertilizers.

The achievements of soil science in increasing crop productivity

British agriculture is a success story and the last 30 years have seen the most rapid transformation in productivity that our farming has ever experienced. Before the 1939–45 war we depended for roughly two-thirds of our food on imports from overseas. Now three-quarters of the kinds of food that can be grown here, or just over 60% of our total requirements, are home-produced on less land, by a smaller labour force, for a larger population. Table 1 gives changes in some crop yields that have occurred in the last century. Increases in yields during the first 67 years from 1885, when statistics were first collected, to 1952 were relatively small; cereal and potato yields increased by only a third. In the last 30 years wheat yields have more than doubled, other yields of cereals and potatoes have increased by over 70%.

A few of the new concepts developed in the last half century, which were reviewed above, have resulted in major advances in our ability to control soil productivity. Their application in the last 30 years has been a major factor in securing yield increases; other factors, such as varieties of crops with greater potential and improved plant protection, are mentioned again later.

Water management

Better understanding of the storage and movement of water in soils, and the use of water by crops, has come from research in the last 50 years. The result has been improved drainage and the spread of scientifically managed irrigation.

Management of crop nutrition

Research during the last 50 years has revealed the agronomic causes of variations in crop responses to fertilizers, provided improved methods of analysing soils and interpreting the results, and has resulted in systems for recommendations to farmers on the use of manures and fertilizers that are scientificially sound and which fit farm practice. The farmers' responses to this advice are shown by the large increases in the amounts of fertilizers used illustrated in Table 1. The need is now to secure greater efficiency in the use of the increasingly expensive nutrients in fertilizers, and particularly in the use of nitrogen

Table 1. Average yields of crops (× 10 kg ha^{-1}) and amounts of fertilizers (× 10^6 kg) used in the United Kingdom for 3-year periods from 1885–1887 to 1980–1982

	1885–87	1911–13	1937–39	1951–53	1961–63	1971–73	1980–82
Crops							
Wheat	2.0	2.2	2.3	2.9	3.8	4.3	6.0
Barley	2.0	1.9	2.1	2.7	3.4	3.8	4.3
Oats	1.6	1.7	2.0	2.4	2.7	3.8	4.3
Potatoes	15	15	18	20	22	29	34
Sugar beet	–	–	21	28	31	39	39
Tomatoes (under glass)	–	–	84	84	86	109	148
Oilseed rape	–	–	–	–	–	2.1	3.1
*Fertilizers**							
Nitrogen, as N	35	30	60	212	475	920	1340**
Phosphorus, as P_2O_5	100	180	170	367	457	487	446
Potassium, as K_2O	4	20	70	218	426	426	461

* Data for the first three periods, up to 1937–39, are approximate estimates

** These data are for the 3-year period 1979–1981.

of which 20 times as much is now applied as was in fertilizers 40 years ago.

Management of cultivations

The work initiated in the 1930s has led to a much better knowledge of the role of cultivations in production systems. The application of this knowledge has been through the increased power provided by tractors, and through better ploughs and implements such as subsoilers and power-harrows, intelligently used. Systems that save energy and avoid unnecessary disturbance of soil structure have also been developed by minimizing cultivations; some areas are direct-drilled with no cultivations at all. No doubt we will hear more of this work in the lecture by Dr Lynch.

Management of soil resources

Progress in soil classification and surveying since the 1930s has been described above. The maps now available have aided the transfer of information from experimental work to farmers' land. The surveys are applied in forecasting deficiencies or excesses of elements that affect plant and/or animal growth, in making fertilizer recommendations, in planning irrigation and drainage systems, in assessing the suitability of land for direct drilling, in planning to use slurries of animal wastes, and for other purposes such as land use planning.

Biological management

Inoculation with appropriate strains of Rhizobium bacteria led to the successful cultivation of legumes, such as lucerne, in areas where the crop had not been grown before. Research on the partial sterilisation of soils has benefitted horticultural crops. Studies of some soil-borne pests and diseases have shown the benefits of crop rotation and the gains from breeding less-susceptible varieties. The management of crop residues, and of manures made from crop and animal wastes, to conserve plant nutrients and recycle them more efficiently, has been aided by microbiological work on composting, on changes occurring in making and handling manures, and on decay in soil.

Whole crop management

The gains in productivity achieved in the last 30 years are the overall result of many changes in crops and their management. Much of the gains in cereal yields has been made possible by breeders providing varieties with potentials to give higher yields and which have been bred with shorter straws to respond to more nitrogen fertilizer without

lodging, and to resist disease. This work of the breeders has been supported by research in other disciplines. Agronomists have devised better ways of growing the crops with improved machinery. The range, and the effectiveness of plant protection materials has greatly increased; under favourable conditions all diseases and pests of leaves and stems can be controlled; only some soil-borne diseases defy control except by crop rotation – an important point for this Conference.

The role of soil scientists in these improvements has been to ensure appropriate soil management, with drainage and irrigation where needed, and the adequate nutrition of the larger crops now possible. This has mostly been done by applying knowledge of the value of manures, fertilizers and lime, and the effects of farming systems on reserves of nutrients and organic matter in soils. All these topics are well-recognised and had been the subject of research 30 and more years ago. Later work has refined our knowledge and capabilities, and aided understanding. New chemical and physical techniques for measuring soil properties and plant composition, developments in optical and electronic instruments, and latterly in computers, have all made our research more effective.

The future contributions of soil science to crop improvement

The question that now arises from the last section is – are we on a plateau of achievement in our application of soil science to crop production? If so, we must look further ahead and ask where the new openings may be for us to give further assistance to agriculture. I believe that these opportunities will arise when we integrate more closely the separate disciplinary attitudes that make up soil science. In particular we must move away from the domination of soil science by chemistry that has existed since Davy's time. We must find a full place for soil physics to interact with other sciences and we must integrate physical and chemical work with biology – because agriculture is essentially the management of living organisms. Up to now we have given full attention to the above-ground parts of plants – which we can see and measure; now we must work much more on the living associations below ground – between plant roots and the macro- and micro-flora and fauna of the soil – which we cannot see, and where work is so much more difficult.

Should we look over the field of soil biology and try to identify the areas where new initiatives may bring a reward? This Conference is a good occasion for reviewing achievements and assembling ideas and integrating them into new research programmes. I will avoid

further discussion of the programme of this meeting, or of current research, but I will now ask some questions about cooperative work that may lead to discussions here or perhaps to further research.

Cooperation with microbiologists

These first questions are related to the associations between roots and micro-organisms which we wish to encourage because they may bring large benefits in improved nutrient uptake and crop growth.

Do we know sufficient about the effects of crops on soil micro-organisms? Can we improve the populations by changes in crop sequences, or mixed cropping?

Should we review again the old idea of inoculating soil with beneficial bacteria? If so we might ask the genetic engineers to design organisms that are nearer to our purpose. We would also need to consider whether the technology of adding bacteria to soil is sufficiently well established, or whether more practical field work is required.

Do we know enough about the nutrients needed by micro-organisms? This is an important question if we plan to encourage populations of beneficial organisms, particularly in soils of low fertility.

Cooperation with plant breeders

Micro-organisms that benefit crops through a symbiotic association that provides extra supplies of N or P or other plant nutrients, receive some of the products of photosynthesis by the host plants through the roots involved in the association. Can we breed varieties of plants that will supply extra amounts of carbohydrates, or other nutrients, to the rhizosphere population?

This last question is one aspect of the general need to cooperate closely with plant breeders. Much breeding is now directed towards developing varieties with resistance to the stresses imposed by soils – notably the aluminium toxicity that occurs in acid soils, nutrient deficiency, and drought. Should we ask for plant selections that develop more beneficial associations with the soil population, perhaps through secretions from the roots, perhaps from different root morphology? These may become very important questions if it becomes possible to associate N-fixing organisms with the roots of cereals and grasses. Breeders and microbiologists already work together to match Rhizobium strains to legume strains; should this be a general objective with other organisms?

Cooperation with physicists

Biologists must regard soil as a cellular material because the voids within the structure provide the habitat for plant roots and other soil inhabitants. Some creatures create the soil structures they require, termites are my example; they create fungal 'gardens' within their mounds by arranging the porosity of the structure so that the air and moisture supplies to their gardens are regular and optimal. Could we ask the physicists to become as competent as the termites and to define desirable soil porosity in terms that aid communication and collaboration with other soil scientists? We know that micro-organisms can create good soil structure. Cooke and Williams[4] encountered a good example of such benefits resulting from growing lucerne at Saxmundham; the roots of the lucerne contained 25% of soluble carbohydrates in their dry matter – good food for micro-organisms when it leaks out of the root. Skinner[14] has described how anaerobic organisms, suitably nourished, release gases that expand soil pores and then stabilise the improved structure so created by lining the pores with bacterial polysaccharides which allow the expanded structure to persist on drying. Should we further pursue such work on structural improvement brought about by micro-organisms?

Cooperation with agronomists

As the possibilities for exploiting root associations with micro-organisms advance we must develop management capabilities to match. This will entail collaboration with agronomists, perhaps in methods of growing crops or introducing special varieties or the use of mixed cropping, perhaps in methods of supplying inocula by mixing it with other materials – such as inert fertilizers like rock phosphate or limestone – that are being added to the soil. Such technologies may become very important in the developing countries and whatever methods are developed should be suitable for application in these countries.

The effective management of the nitrogen cycle is a much greater challenge to agronomists than is posed by the management of any other nutrient or group of nutrients. Although the work required on nitrogen is great in volume, and is difficult, the returns from success in terms of extra crop growth from improved management will be great. There is much scope here for cooperation between agronomists and microbiologists. We must take every opportunity of capturing more nitrogen from the air and of preventing losses from the agricultural system. While most of the effort will go into the nitrogen cycle, which will receive full attention here in Reading, we must not forget other cycles, for example those involving carbon, phosphorus

and sulphur. I must ask – why do we so often ignore the sulphur cycle? Micro-organsisms are much involved in changing the state of combination of sulphur and they cause losses from the system by the evolution of hydrogen sulphide and by the release of sulphate which may then be leached. Sulphur deficiency is already a serious problem in many developing countries, and it will increase in some temperate regions as experience in Ireland has already shown[2]. Sulphur will become a more important component of fertilizer recommendations as more concentrated fertilizers containing no S are used, and as compaigns to remove SO_2 from industrial emissions and products of combusion are successful so that the atmosphere supplies less sulphur to our crops.

Other initiatives

I will not intrude further on the subjects of the speakers invited to give keynote and introductory papers on the main themes of this Conference, but I will conclude this section by mentioning three topics that do not appear in the programme; I think they are important when considering the benefits that may arise from improved management of the associations between roots and micro-organisms.

Biological control of root pathogens Some of the micro-organisms found in soil are antagonistic to bacteria that are plant pathogens. If these antagonistic organisms could be introduced, or the system could be manipulated to favour them, biological control of the plant disease could become very effective and economic. This important subject was discussed briefly by Brown[3] and by Garrett[6].

Production of plant-growth hormones Soil micro-organisms produce small amounts of plant-growth regulating substances and these may have marked effects on plant performance. Again, if the population could be manipulated to favour these particular organisms, there could be practical gains from improved crop growth. Brown[3] has discussed the subject.

Allelopathy Pickering[9] initiated work on this topic by showing how one plant could affect another species growing nearby, and how one plant could affect its successor on the same site. The early literature was reviewed by Ripley[11] but little interest was taken in the subject until the last few years when the name I have used was given to the damage that one plant does to another; Rice[10] has written on the topic. Root exudates are very important through the effects they

have on life in the soil; they interact with other soil constituents, with other roots, and with micro-organisms. With a better understanding, which should be achieved by cooperation with biochemists, root exudates may become one of the tools in the management of soil fertility.

Conclusions

To make further progress through applying soil science to crop production we must integrate knowledge of chemical, physical and biological processes occurring in soil into systems for controlling soil productivity. Success will be judged by the practical benefits we provide. Joint research by all three disciplines in the region where roots meet soil will be essential. Agronomists must join us to define the ideal structure of soil for root systems to grow unimpeded and to establish beneficial rhizosphere populations, while obtaining optimum amounts of water and nutrients. We must then work together to create these ideal structures.

As our understanding of the conditions and processes in the rooting zone of the soil deepens it will be possible to plan better for fertility management practices that realise the full potential of a crop. We must develop the ability reliably to reproduce optimum conditions despite climatic vagaries; competent soil management will avoid the surprises that are now so common when we fail to obtain sufficient information to ensure that errors do not occur as we seek to achieve the potential yield of a crop. Man's abilities in science and engineering depend on a full knowledge of the properties of materials and of the processes and systems that are involved, for example in a venture such as a flight to the moon. We must plan to achieve the same competence through knowledge of the properties of our soils, the transport processes that supply nutrients and water to roots, and particularly of the whole ecology of the living world beneath the soil surface. Then we will become the reliable controllers of soil productivity, and therefore of man's destiny.

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Section 1

Cycling of nitrogen in soil-plant systems

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Cycling of nitrogen in modern agricultural systems

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Key words Agriculture Barley Budgets Fauna Fertilizers Global cycles Lucerne Meadow fescue Microorganisms Nitrogen

Summary Agro-ecosystems have developed from mixed- and multiple-cropping systems with relatively closed N cycles to intensively managed monocultures with large N inputs in the form of commercial fertilizers. Cultivation of increasingly larger areas of land has resulted in substantial losses of soil organic matter and N. Also, the move from slash and burn agriculture to intensively ploughed systems has resulted in losses through increased erosion.

The use of N fertilizers has increased rapidly to *ca.* 60 Tg N yr^{-1} (1980/81), which is equivalent to at least 40% of the N fixed biologically in all terrestrial systems and 36% more than is fixed in all croplands. On a global scale, the major losses of N from agro-ecosystems are estimated to be: harvest, 30 Tg; leaching, 2 Tg; erosion, 2–20 Tg; denitrification 1–44 Tg; and ammonia volatilization, 13–23 Tg. However, the data base is very crude and several estimates may be wrong by as much as one order of magnitude.

Additions of N fertilizers have both direct and indirect effects on soil microorganisms. The possible importance of such effects is briefly discussed and a specific example is given on long-term effects on soil microbial biomass and nitrification rates in 27-year-old cropping systems with different N additions: (i) 0 kg N ha^{-1} yr^{-1}, (ii) 80 kg N ha^{-1} yr^{-1}, (iii) farmyard manure *ca.* 80 kg N ha^{-1} yr^{-1}.

Few detailed N budgets exist for agro-ecosystems, despite its major importance as a limiting plant nutrient and the large losses of N from such systems. In conclusion, preliminary nitrogen budgets for four cropping systems (barley receiving 0 or 120 kg N ha^{-1} yr^{-1}; meadow fescue ley with 200 kg N ha^{-1} yr^{-1} and a lucerne ley) are presented, with special attention to N flow through the soil organisms.

Introduction

Modern agriculture has its roots in small scale farming originally developing in a patch-wise fashion around the globe. Harlan *et al.*[25] considered traditional agriculture in Africa to have developed from a mosaic of crops, traditions and techniques: this development originated wih nomadic herding followed by different types of shifting cultivation, bush fallowing and slash-burn agriculture[33]. Such traditional farming systems could be remarkably productive when, through trial and error, they were maintained in ecological balance[22]. However, as exotic crops were introduced new cropping systems and practices evolved globally. Maize, potatoes, cassava, papaya, groundnuts and cocoa came from South America; soybean, rice, wheat, bananas and citrus fruits from Asia; coffee and sorghum from Africa; oats and rye from Europe[24].

Increased pressure on available land for food and fodder production

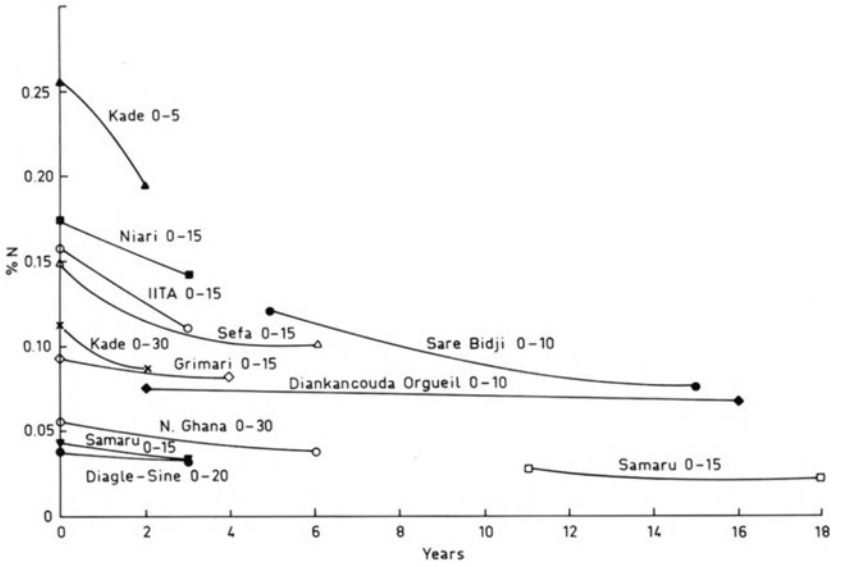


Fig. 1. Changes in soil nitrogen contents after clearing from natural vegetation of some West African sites[23].

changed the shifting cultivation from the original pattern with a fallow period often more than ten times as long as the cropping period, to compound farming and intensive subsistence agriculture, with a fallow period shorter than the cropping period[33]. Clearly this change is from ecologically sound to ecologically imbalanced systems[40], so it is possible to distinguish between traditional shifting cultivation and shifting cultivation in disequilibrium[47].

Intensified cropping of land with shortened fallow periods led to N losses from the systems (Fig. 1) and decline in productivity consequent on more rapid soil organic matter decomposition and increased wind and water erosion. In areas such as the corn belts of Canada and the United States, soil N contents have decreased rapidly and wind erosion during the 1930s resulted in severe agricultural problems.

Fortunately N is an abundant element and by the late 1800s man had learned to fix dimolecular nitrogen chemically in forms available to crops. Production of commercial N fertilizers has probably been the single most important factor resulting in dramatically increased crop yields, especially in industrialized countries.

Nitrogen fertilizer consumption

Modern high yielding agricultural systems depend on either the addition of N fertilizer, organic N (*i.e.*, manure) or an efficient

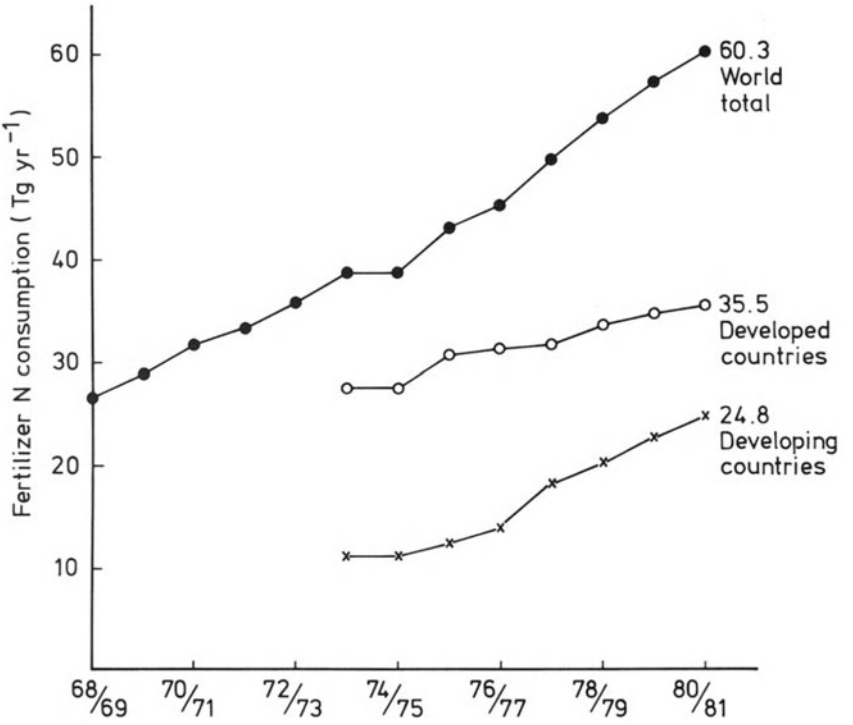


Fig. 2. World use of nitrogen fertilizers (Tg N yr^{-1}) 1968/69–1980/81. For the period 1973/74–1980/81 the use has been separated between developed and developing countries according to FAO[16]. The figures show total fertilizer use in Tg for the year 1980/81.

Rhizobium-legume symbiosis. In spite of the rapid increases in energy prices during the 1970s, there has been a steady increase in the use of N-fertilizers (Fig. 2), except for a minor stagnation in 1975. This increase was not restricted to the affluent industrial countries, and in fact the rate of increase over the past ten years has been higher in developing than in developed countries, the annual increases for 1973/74–1980/81 being 15 and 4%, respectively. In 1980/81 the developing countries accounted for 41% of the global consumption of N fertilizers and by the end of the century as much fertilizer will be used in developing as in developed countries.

The consumption of 60 Tg fertilizer N in 1980/81 can be compared with the estimated 44 Tg N annually fixed in all croplands[9]. With the present rapid increases in the use of fertilizers, the amount of combined N added to croplands by man will probably equal the amount of N fixed biologically in all terrestrial ecosystems by the end of this century[43].

The use of N fertilizers over the globe is very uneven and both the

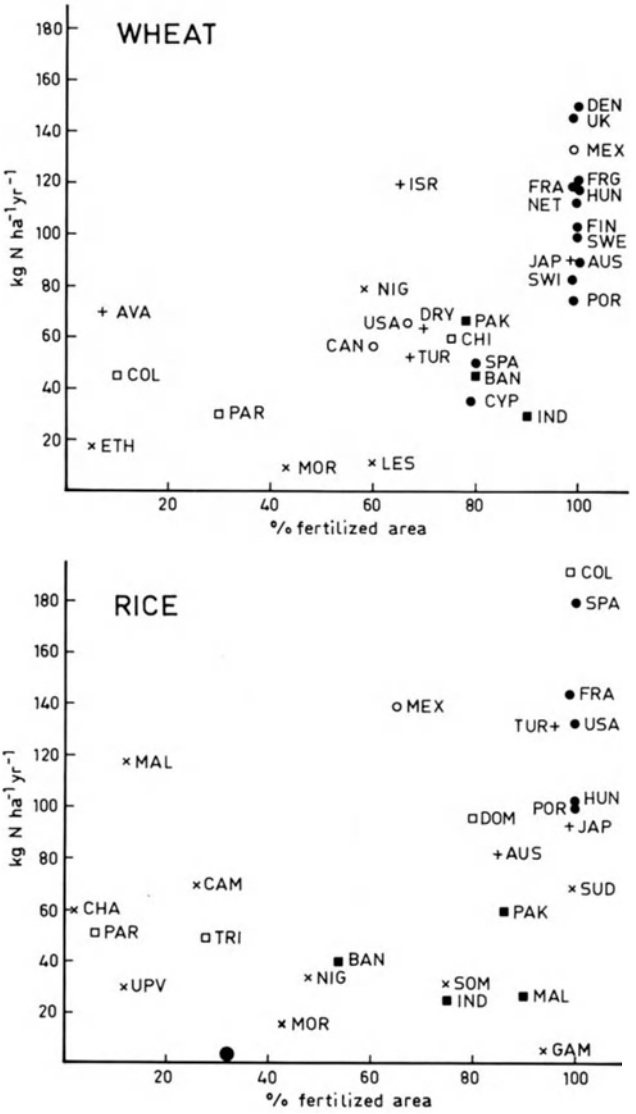


Fig. 3. Fertilizer N use for wheat and rice in selected countries with the rate (kg N ha^{-1} yr^{-1}) plotted against percentage of cropped area fertilized. Data from Martinez, Diamond (1982). The following symbols indicate geographical areas: X = Africa, ○ = North America, Mexico, □ = South America and Caribbean, ■ = South Asia, ● = Europe, + = other.

percentage of harvested land fertilized and the rates of application vary considerably as is exemplified for wheat and rice in Fig. 3. In general, West European countries apply large amounts and almost all harvested lands are fertilized. In contrast, for wheat in Ethiopia only 5% of the land is fertilized, with an average of 18 kg N ha^{-1} yr^{-1}.

Table 1. Global harvested areas for some major crops (FAO[17]) and N application rates for fertilized land in specified countries (Martinez and Diamond[31]; de Geus[21]). Total consumption of fertilizer N if all cropped areas received the specified rates

Crop	Global harvested area (10^6 ha)	Fertilization rate (kg N ha^{-1} yr^{-1})	Country	Total need for fertilizer (Tg N)
Wheat	237	130	Mexico	30.8
Paddy rice	144	140	Mexico	20.2
Maize	129	150	US	19.4
Barley	79	90	Mexico	7.1
Pulses	62	40	Mexico	2.5
Sorghum	45	85	US	3.8
Millet	43	130	Egypt	5.6
Cotton	33	120	Mexico	4.0
Oats	25	100	Mexico	2.5
Potatoes	18	190	UK	3.4
Rye	17	70	Sweden	1.2
Cassava	14	60	(de Geus)	0.8
Sugar cane	13	210	US	2.7
Sweet potatoes	12	60	(de Geus)	0.7
Sun flower	12	(100)	–	1.2
Rapeseed	11	260	UK	2.9
Grapes	10	100	France	1.0
Coffee	10	160	Colombia	1.6
Sugarbeets	9	140	Sweden	1.3
Cocoa	5	60	Mexico	0.3
Tobacco	4	32	Tanzania	0.1
Jute	3	30	Bangladesh	0.1
Tea	2	90	Tanzania	0.2
Total	937	$\bar{X} = 120$		113.4

There is also a clear tendency for some countries to concentrate fertilizer use on relatively small areas. For example, only 7% of wheatland in Australia is fertilized but the rate is 70 kg ha^{-1}, whereas in Lesotho as much as 60% is fertilized but the average rate is only 11 kg ha^{-1}. For rice, only 12% of the land in Mali is fertilized at an average rate of 118 kg ha^{-1}, while in Gambia 94% of the paddy rice is fertilized but the average rate is only 6 kg ha^{-1}. It should be kept in mind that the data base may be somewhat unreliable.

Fertilizers are needed in order to achieve maximum yield[12]. In developing countries FAO has mounted a massive set of field trials to determine how to maximize yields and to demonstrate to farmers the importance of fertilization. Yet, even if farmers are convinced of the beneficial effects of plant nutrient additions, they often cannot afford the use of fertilizers, in spite of common large government subsidies.

If all wheatlands (237×10^6 ha) received as much N fertilizer as is

Table 2. Losses of N from the global agro-ecosystems

Process	Rate (Tg N/yr)	Certainty of estimate
Harvest	30[a]	(+ + +)
Leaching	2[b]	+ +
Erosion	2–20[c]	+ +
Denitrification	14–132[d]	+
	1–44[e]	
Nitrification (N_2O)	10[f]	+
NO + NO_2 (NO_x)	0.4–2[g]	+
Ammonia volatilization	13–23[h]	+
Burning	?[i]	(+)

Most figures are based on slender data. The relative certainties of the extrapolations are also given (+ + + = certain within 10–50%; + + = certain within 50–100%; + = uncertain, may be wrong by at least an order of magnitude).

Notes

[a] The world crop production in 1965 was 1882×10^8 kg protein equivalents[29]; protein contains 16% N so this would equal 30 Tg N. *cf.* fertilizer use of 20 Tg in 1965 and a N fixation of *ca.* 44 Tg in all croplands[9]. Fertilizer use in 1980/81 was 60 Tg N, but crop output has probably not trebled.

[b] Anthropogenic flux to the oceans is 7 Tg/yr according to Meybeck[32]. The proportions of this coming from agricultural areas and from sewage are not known. For Sweden an average of 29% of river N transport originates from agricultural drainage[2].

[c] 10–100% of the total amount of particulate matter N in river runoff as given by Meybeck[32].

[d] Extrapolated from data in Söderlund and Svensson[43] with N_2O losses from agro-ecosystems being 13–82% of global losses and global total denitrification losses from terrestrial ecosystems being 107–161 Tg N/yr.

[e] Extrapolated from Rosswall[37] giving denitrification losses of fertilizer N as 2–73%.

[f] Based on a mean rate of 0.4 kg/ha of N_2O-N evolved over 20 days[8], and an agricultural area of 14×10^{12} m^2 (Söderlund and Svensson[43]), further assuming an equal loss rate throughout the year.

[g] Extrapolated from an average NO_x-N production of 1–5 ng/m^2 (Rosswall *et al.*[39]) and an agricultural area of 14×10^{12} m^2.

[h] According to Crutzen[13], 20–30 Tg lost from domestic animals and < 3 Tg from arable land.

[i] Crutzen[13] estimated N losses from biomass burning as follows: NO_x 10–40 Tg/yr, N_2O 1–2 Tg/yr, NH_3 < 60 Tg/yr. The amount of this which is accounted for by burning of agricultural residues and pastures is not known.

presently used on wheatlands in Mexico (130 kg ha^{-1} yr^{-1}), over half the present consumption would have to be used for wheat alone (30.8 Tg yr^{-1}), while the total demand for all other major crops would be twice the amount used today (Table 1). In addition, large amounts of N fertilizers are consumed on grasslands used for grazing or harvest of ley crops. FAO has estimated a need for at least a 40% increase of harvested land in less developed countries by the year 2000, which would call for at least a similar increase in fertilizer use. To summarize in the words of Edouard Saouma, Director-General of FAO, in his report "**Agriculture Towards 2000**" (FAO, 1981[15]): "By year 2000 a world population of more than 6 billion will require an agricultural

output some 50–60% greater than in 1980. Demand for food and agricultural products in developing countries will double. It is essential, within the next few years, to take many of the decisions which will determine the world food situation in 1990, or even in 2000".

Nitrogen losses from the global agro-ecosystem

Even though the use of N fertilizers has increased rapidly, there have not yet been any marked detrimental environmental effects on a global scale, although local and sometimes regional problems are becoming increasingly evident. In Table 2, estimates of N losses for all agro-ecosystems are given as an indication of magnitude only.

The global amount of N removed by harvest is about 50% of that added through fertilizers. This also applies on a regional level, as can be seen, for example, from the N budget for Swedish agriculture[27]. It is probable that utilization efficiency will decrease as application rates increase. There are great variations between regions, cropping systems, application rates and types of fertilizer used, so it is dangerous to use this rule of thumb for individual systems. Suffice it to say that crop utilization is generally low.

From a global point of view, leaching does not seem to be a major loss process (Table 2). However, this may be a false impression, since the estimate is based on N dissolved in river runoff and does not take into account the immediate loss of NO_3^- leached below rooting depth. The increasing amount of NO_3^- in groundwater is a more direct measure of leaching losses. NO_3^- leaching into groundwater used for human consumption is a problem not only in Europe and the United States, but also in developing regions, especially those with high rainfall and intensive agriculture. In these regions NO_3^- levels reach concentrations far above those given by the WHO as safe limits for human consumption (50 ppm NO_3^-). In some areas of Sri Lanka the NO_3^- content of groundwater have reached 200 ppm (K. Theivendirarajah, pers. comm.). Better fertilizer and water management practices are urgently needed to alleviate these problems.

Erosion losses of N are increasing especially in tropical countries with torrential rain-storms, and increasing pressure on land which forces farmers to utilize marginal land on steeper slopes. The changes in cropping practices from long fallow periods to continuous cropping with decreased mulching and intensified cultivation increases the risk of erosion. The spread of no-tillage agriculture will help conserve valuable topsoil from wind and water erosion but this practice introduces large amounts of herbicides, which is another cause for concern.

Although field measurements often show denitrification to be of minor account in N losses from agro-ecosystems, an overview of estimates for numerous cropping systems showed a range from 2–73% of the added N[37]. Observations of N_2O turnover times in the atmosphere also indicate larger N losses than those observed through field measurements for denitrification, as do attempts to construct N budgets for agro-ecosystems either on the global, regional or local scale. Failures of field measurements to indicate denitrification as a major loss process may perhaps be a result of pulse events following rainfall.

Recent observations of N_2O loss after the application of urea and ammonium fertilizers have shown that the nitrification process can also lead to gaseous losses of added fertilizer N[8]. It has still to be shown that this loss is of agronomic importance, but certainly it has to be taken into account in attempts to estimate the total N_2O emissions from agricultural land to the atmosphere.

NO can also be produced during both ammonium oxidation[30] and nitrate reduction[19] but limited field studies indicate that denitrification mainly accounts for the observed production from arable soil[39]. Such losses are certainly not of agronomic importance, but must be considered when estimating NO_x ($NO + NO_2$) pollution of the atmosphere.

NH_3 volatilization can be appreciable, especially after spreading animal manure and sometimes after heavy applications of urea or ammonium fertilizers[18]. The use of sulphur-coated urea or other slow release fertilizers reduces such losses.

Burning agricultural residues results in large N losses to the atmosphere but at present it is not possible to estimate this loss. However, the practice should not be encouraged, as it leads not only to unwanted losses of an important nutrient but also decreases the amounts of organic matter returned to the soil.

Effects of nitrogen additions on soil microorganisms

N cycling is mainly regulated by biotic processes and microorganisms especially play a crucial role in controlling the fate of N fertilizers[38]. Many studies have been performed to elucidate both short-term and long-term effects of N fertilization on microorganisms. As an example, in long-term 27-year-old field plots at Uppsala, Sweden the relative effects of N additions as fertilizer or manure were compared in annually cropped (mostly cereals) and continuously fallowed treatments (Table 3).

Except for the amount of fluorescein-diacetate (FDA) hydrolysing

Table 3. Effects of continuous annual cropping (mostly cereals) for 27 years compared to a continuously fallowed system. Data are expressed as per cent increase relative to the fallow for three cropping systems (i) no N fertilizer, (ii) 80 kg N ha^{-1} yr^{-1} as calcium nitrate, (iii) 80 kg N ha^{-1} yr^{-1} as manure (also adding *ca.* 1800 kg C ha^{-1} yr^{-1}). Data from Schnürer *et al.*[42], except for nitrification from Berg and Rosswall[7].

	No N	80 kg N ha^{-1} yr^{-1}	
		$Ca(NO_3)_2$	Manure
Soil carbon	14	22	63
Biomass by fumigation	28	75	115
Biomass direct count	63	109	311
Respiration	58	100	226
FDA activity[1)]	33	100	300
FDA active mycelium[2)]	0	19	63
Total mycelium length (Jones and Mollison[28])	51	106	101
Bacteria, direct count	16	41	91
Protozoa, MPN	0	4300	5270
NH_4^+-oxidizers, MPN	192	47	2050
Potential nitrification rate[3)]	23	118	200
Actual nitrification rate[3)]	8	272	199

[1)] Total fluorescein diacetate hydrolytic activity according to Schnürer and Rosswall[41]

[2)] According to Söderström[44]

[3)] Using the chlorate inhibition method according to Belser and Mayes[6] with modifications according to Berg and Rosswall[7].

mycelium (estimated according to Söderström[44]) and most probable counts of protozoa (estimated according to Darbyshire *et al.*[14]), the presence of a crop increased all indices even without N additions. This shows the importance of C additions to the systems, through root production, for the enhancement of microbiological activity in the soil. The importance of an energy supply is further supported by the relatively large increases in most measured indices in the manure treatment compared with the same amount of N added as inorganic fertilizer.

The most dramatic increase was in the numbers of protozoa, which increased 40–50 times in plots fertilized with either inorganic fertilizer or manure. This suggests that bacterial production in those treatments was much higher than indicated by the "standing crop" of bacteria. The importance of bacterial grazers as indicators of bacterial activity has earlier been observed both for nematodes[5] and protozoa[11].

The numbers of ammonium oxidizers (measured by a most probable number method) did not reflect either the differences in potential or real nitrification rates, as determined by nitrite accumulation in the presence of sodium chlorate, with or without ammonium additions.

It is difficult to distinguish the effects of N additions *per se* from

those of increased crop growth resulting in a larger input of organic matter to the soil system. In order to understand the effects of different cropping systems on N cycling, more detailed process studies are needed with a synthesis of this information into N budget/flow diagrams, as will be discussed below.

Nitrogen budgets of four Swedish cropping systems

Input/output N budgets for agricultural systems, based primarily on lysimeter studies, have existed for many years (*cf.* 3). More recent work includes the compilation of balance sheets for model cropping systems from several regions in the world to compare N cycling attributes of different management systems[20]. However, large uncertainties about existing N budgets remain, particularly regarding gaseous losses and the contribution of soil organic matter to N fluxes between plant, organic matter, and soil organisms.

The budgets presented below are our attempt to synthesize some of the information gathered from the first two intensive field seasons of a project concerning N cycling in agro-ecosystems. Our estimates are preliminary and will be further refined as new information becomes available.

Calculation of N flows

Budget calculations are based primarily on 1981 field samplings conducted in four cropping systems: barley receiving no nitrogen fertilizer, barley receiving 120 kg N ha^{-1} yr^{-1} (as calcium nitrate), meadow fescue receiving 200 kg N ha^{-1} yr^{-1}, and a N fixing lucerne crop. The experimental field is situated at Kjettslinge, Central Sweden and the top soil is a loam with pH 6.3 and C and N contents of 2.2 and 0.23%, respectively[46]. Above-ground and root production data in meadow fescue are from 1982, because primary production was not measured in this system in 1981. Both ley crops were initiated in 1981, hence the primary production in lucerne reflects an establishing crop with only one harvest, while the meadow fescue is an established ley with two harvests.

Primary production

Root soil corings and clippings of above-ground vegetation were taken approximately every two weeks from May until harvest (late August) in the barley and until November in the leys. Surface litter in all crops was sampled. Barley root samples were fractionated into live roots and soil litter (dead roots plus incorporated above-ground residue). Meadow fescue and lucerne root cores were not fractionated. Dry weight, ash content and total N content were determined for each fraction.

Above-ground production was calculated by difference for each sampling interval. Calculation of root production was more difficult, since the continual processes of root mortality and root litter decomposition cannot be directly measured. Decomposition rates of root-derived material were estimated from declines in the soil litter fraction measured in the first sampling interval in barley. During this period root mortality was probably small so that the decline reflects the gross decomposition rate. Root growth and mortality were then calculated by difference, correcting for the estimated decomposition during each interval.

Uptake of N by the plant was based on measured N accumulation above-ground and the requirements of the roots, calculated from root growth and measured root N content for each sample period. N turnover via fine root dynamics and root exudates cannot be estimated from the soil core data and has not been included in the budgets.

Soil fauna

Data on soil fauna population dynamics were based on 3 sampling occasions for micro- and macro-arthropods and enchytraeids, 4 for nematodes, 2 for earthworms and 12 sampling occasions for protozoa during 1981. Sampled fauna were divided into taxonomic groups (36 for nematode fauna, 14 for micro- and macro-arthropods, and 3 for protozoa) and further divided into trophic level or feeding groups (herbivore, saprovore, microbivore or carnivore). Respiratory metabolism was calculated for each taxonomic group using an O_2 consumption/body weight relationship with parameter values suggested by Persson *et al.*[36]. Temperate influences on respiration rates were included using a Q_{10} relationship with measured daily soil temperatures and compensating for diurnal temperature fluctuation[1]. Consumption, production and defecation (in terms of C) were then calculated on the basis of respiration, using the energetic coefficients of Heal and Maclean[26]. Using estimated C metabolism, N consumption was determined from C/N ratios of ingested material; and N in biomass production was calculated using C/N ratios for the faunal groups.

Since ammonium and urea are primary excretion products of nematodes and protozoa[4], excreted N from these groups was expressed as a direct flow to the mineral N pool. Non-assimilated N in other faunal groups was recycled through the soil litter pool. Faunal populations were considered to be in steady-state from year to year so that the annual production was recycled to the litter pool as mortality.

Soil organic matter

A portion (30%) of the current year's decomposition of soil litter was transferred to the soil organic matter pool as stabilized material, based on initial results from humification studies in the project (J. Persson, pers. comm.). Soil organic matter C-mineralization under barley was based on turnover times for soil organic matter fractions estimated in long-term field experiments with wheat[34]. N flow was then calculated from the C flow, assuming the C/N ratio of the soil organic matter pool remained constant. Since corresponding data on soil organic matter changes under a ley crop were not available, N mineralization from this pool in the leys was calculated by balance.

Soil microbial flows

Inputs of N to the soil microorganisms was estimated from the decomposition calculations. Net mineralization was calculated as the difference between inputs to the microorganisms and outputs due to consumption by soil fauna.

Inorganic nitrogen inputs and outputs

Levels of NH_4^+ and NO_3^-N were based on soil samples from 9 occasions during the year. The decline in inorganic N, indicated by the delta symbol, represents the measured difference in the inorganic N pool from January 1981 to January 1982. Large amounts of inorganic N ($15\,g\,m^{-2}$, 0–1 m) existed in all treatments at the start of 1981 following the first year's installation of drainage and initiation of intensive cropping. Atmosphere deposition, NH_3 volatilization and leaching are measured values. Denitrification, in the barley treatments, was calculated by balance. Denitrification in the leys was based on calculated rates in the barley treatments and the relative differences in denitrification rates between the cropping systems measured in September 1981.

Annual N budgets were prepared using the data and methods described above. Fig. 4 shows an example of a complete budget, with the exception that the nematode compartment is condensed from root consumer, fungivore and bacterivore compartments. The microbial compartments are framed with a dashed line indicating that several of the flows do not differentiate between bacteria and fungi. Flows indicated by question marks have not been

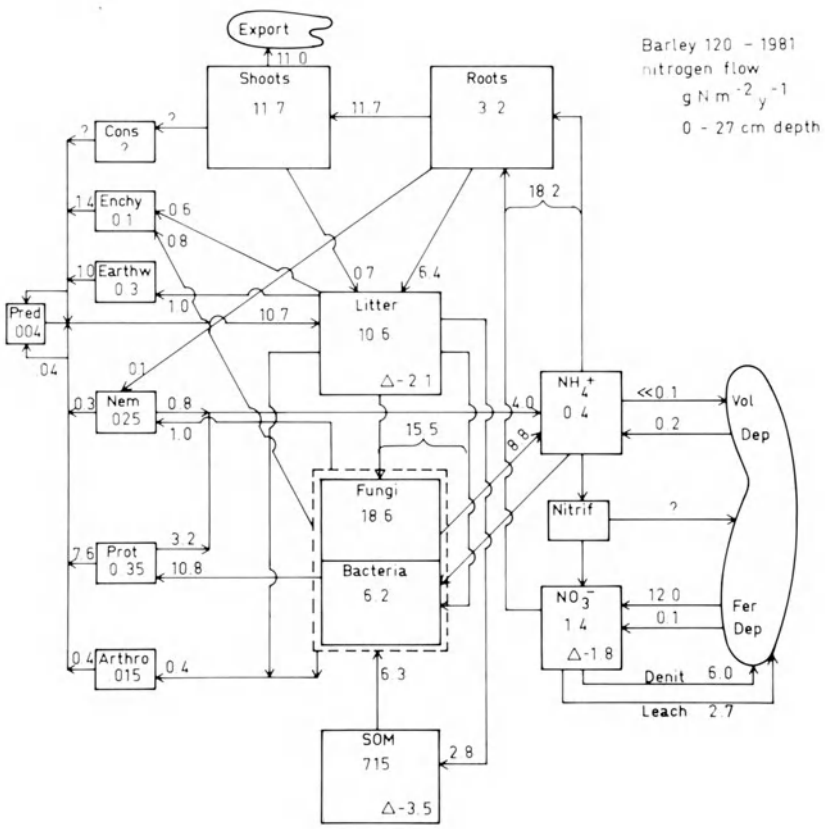


Fig. 4. Budget for an annual barley crop receiving 120 kg N ha^{-1} yr^{-1} as calcium nitrate. Compartment abbreviations are as follows: cons (above ground consumers), enchy (enchytraeids), earthw (earthworms), pred (predatory fauna), nem (nematodes), prot (protozoa), arthro (soil micro- and macro-arthropods), som (soil organic matter), nitrif (nitrifying bacteria), vol (volatilization), dep (dry and wet deposition), fer (fertilizer), denit (denitrification), leach (leaching). Compartment values (g N m^{-2}) are mean annual estimates except for shoots and roots which are peak standing crop values. Delta symbols indicate net changes, otherwise compartments are assumed to be in steady-state. All flows are expressed as g N m^{-2} yr^{-1}. Question marks indicate flows which were not estimated.

estimated and blank arrows indicate flows which were not separately estimated (*i.e.* nitrification, microbial immobilization) but which are included in net compartment flows.

Results and discussion

Summarized N budgets for each of the four cropping systems are shown in Fig. 5a–d. As in most arable soils, the N is mainly present in soil organic matter and litter, and annual N fluxes through the soil microorganisms represent only a small fraction of the total soil N, from *ca.* 5% in the ley crops to *ca.* 2.5% in the unfertilized barley.

Table 4. Inputs and outputs from four cropping systems on a loamy soil at Kjettslinge, Central Sweden (Steen *et al.*[46]): Lucerne ley with no N-fertilizer, meadow fescue grass ley receiving 200 kg N ha^{-1} yr^{-1} as calcium nitrate, barley receiving 120 kg N ha^{-1} yr^{-1} and barley without N (Steen *et al.*[46]). All data are given as g N m^{-2} yr^{-1}; data are preliminary, and details of calculation methods are given in the text

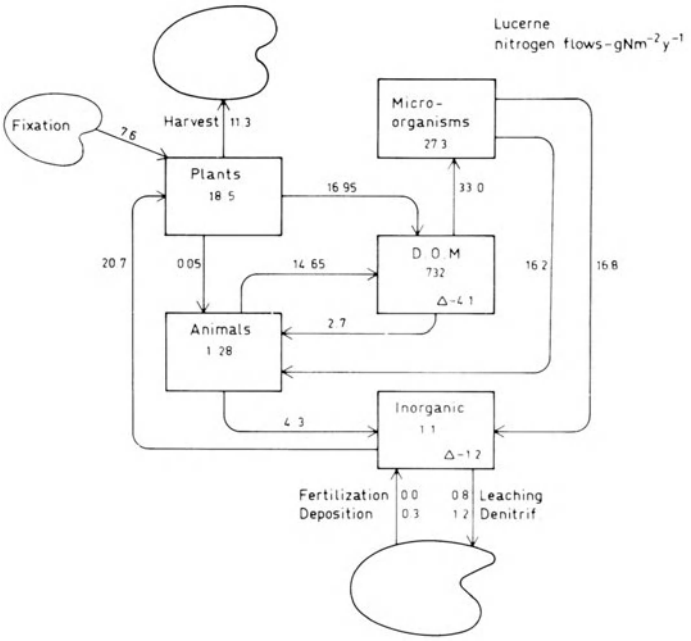
Cropping system	External input (EI)	EI + mineralization	Harvest	Leaching	Denitrification	N loss index (%)
Lucerne	7.9	29.0	11.3	0.8	1.2	6.8
Meadow fescue	20.3	46.0	23.2	1.7	3.7	11
Barley 120	12.3	24.7	11.0	2.7	6.0	35
Barley 0	0.3	10.8	3.7	2.1	3.1	47

Table 5. N mineralization and total soil organic matter N balance for four cropping systems: Lucerne ley with no N-fertilizer addition, meadow fescue grass ley receiving 200 kg N ha^{-1} yr^{-1} as calcium nitrate, barley receiving 120 kg N ha^{-1} yr^{-1} and barley without N. Data are preliminary and details of calculation methods are given in the text

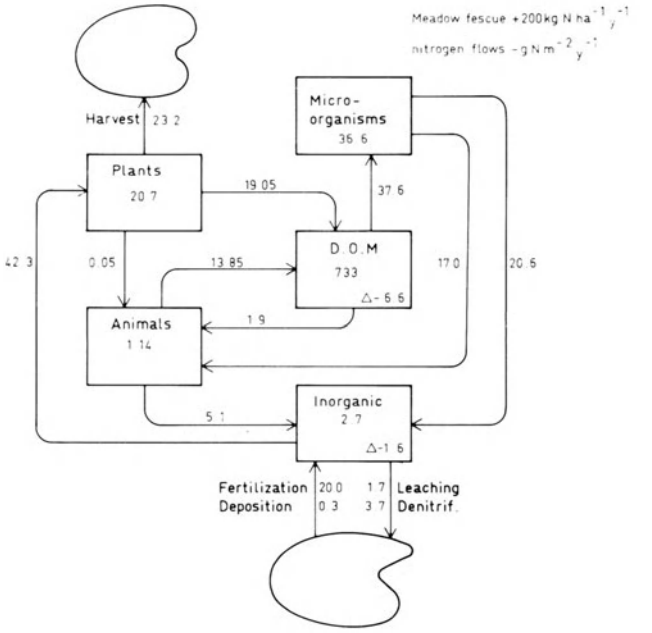
Cropping system	Estimated net mineralization (g N m^{-2} yr^{-1}) I	Relative mineraliz. (% of soil N) II	Mineralization via fauna (% of I) III	Total soil N balance (g N m^{-2} yr^{-1})
Lucerne	21.1	2.8	20	– 5.3
Meadow fescue	25.7	3.4	19	– 8.2
Barley 120	12.8	1.8	31	– 7.4
Barley 0	10.5	1.4	36	– 8.7

N inputs and outputs vary widely between systems (Table 4). The major loss pathways are leaching and denitrification. NH_3 volatilization losses were less than 1 kg ha^{-1} yr^{-1} and NO_x losses are also small. Total N losses were greatest in the fertilized barley and least in the lucerne ley. The proportion of total N loss (excluding harvest) due to denitrification was fairly constant for all treatments, between 60 and 68%. The concept of a N loss index (Table 4) was formulated as a measure of the N retention ability of the system. Total non-harvest N losses were divided by the sum of external inputs of inorganic N and the estimated net mineralization; external N input plus mineralization can be considered as a measure of the potential availability of N to loss pathways. The continual presence of a plant cover in the leys acting as a sink for inorganic N, particularly during spring and fall periods when potentials for losses are high, is the most important reason for the low N loss indices in the leys compared to the barley crops. In fact, leaching losses of N were higher from the barley system without additions of fertilizer N than from the grass ley receiving 200 kg N ha^{-1} ha^{-1}.

Net mineralization of soil organic N in the ley crops was about twice as high as in the barley treatments (Table 5). Stanford and


Lucerne
nitrogen flows - gNm⁻²y⁻¹
Fixation
7.6
Harvest 11.3
Plants
18.5
Micro-
organisms
27.3
16.95
33.0
20.7
0.05
14.65
D.O.M
732
Δ-4.1
16.2
16.8
Animals
1.28
2.7
4.3
Inorganic
1.1
Δ-1.2
Fertilization 0.0 0.8 Leaching
Deposition 0.3 1.2 Denitrif.

a


Meadow fescue +200kg N ha⁻¹ y⁻¹
nitrogen flows - g N m⁻² y⁻¹
Harvest 23.2
Micro-
organisms
36.6
Plants
20.7
19.05
37.6
42.3
0.05
13.85
D.O.M
733
Δ-6.6
17.0
20.6
Animals
1.14
1.9
5.1
Inorganic
2.7
Δ-1.6
Fertilization 20.0 1.7 Leaching
Deposition 0.3 3.7 Denitrif.

b

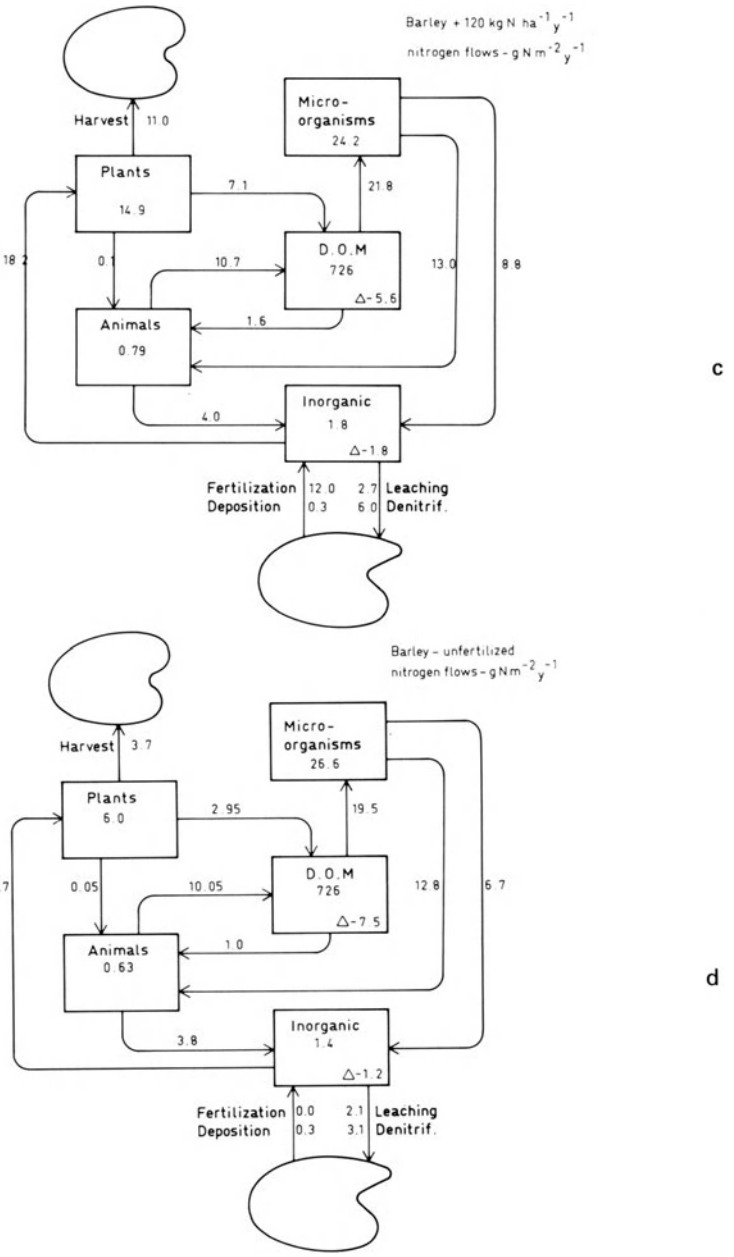


Fig. 5. Summarized budgets for four cropping systems: a) lucerne ley without N-fertilizer, b) meadow fescue ley receiving 200 kg N ha^{-1} yr^{-1} as calcium nitrate, c) barley receiving 120 kg N ha^{-1} yr^{-1} and d) barley without N. Compartment values are in g N m^{-2} and represent peak standing crop for plants and mean annual values for all others. Delta symbols indicate net changes, otherwise compartments are assumed to be in steady-state. Flow values are in g N m^{-2} yr^{-1}. D.O.M. = Dead organic matter.

Smith[45] reported a 30% higher potential mineralization in U.S. cereal rotations containing leys compared to continuous cereal cropping. One explanation for this increased mineralization may be that high root-C input to the soil in the leys enhances the release of N from soil organic matter, as postulated by Clarholm[10]. The decomposition of above-ground material with relatively high N contents in the leys also contributed to the high mineralization rates. It should be noted that N mineralized amounts are high compared to those reported in N budgets for similar arable systems[20]. However, these values are often calculated from above-ground uptake of N in unfertilized plots, and N accumulation in below-ground plant biomass is not considered. Table 5 shows a net loss of soil organic N in all treatments including those receiving high fertilization. The installation of tile drainage and the initiation of intensive cropping at the start of the field experiment in 1979 after one year in fallow may be a reason for the high mineralization rates and net loss of N even from the fertilized treatments.

The soil fauna appears important in releasing mineral N through the grazing of microbial biomass with a low C/N ratio. Only the protozoa and nematode faunas were estimated to excrete significant amounts of mineral N. Mineralization directly via the soil fauna was highest in the leys, amounting to over $5\,g\,N\,m^{-2}\,yr^{-1}$ in the meadow fescue ley (Table 5). However, the proportion of total mineralization attributed to the fauna was highest in the unfertilized barley and lowest in the leys. For a N-poor pine soil, Persson[35] calculated that 30% of total net mineralization was due to faunal activity. The increasingly important role of the soil fauna in mineralization as the system becomes poorer in N seems reasonable in view of the increase in immobilization by microorganisms as the N contents in the substrates decrease.

Conclusions

Our quantitative knowledge of the fate of N in agro-ecosystems is still unsatisfactory. Although the importance of crop yield as a function of fertilizer input has been well documented, reliable quantifications of N losses are urgently needed. An understanding of the relative importance of different loss processes is essential in order to design management practices, which will be more efficient in conserving nitrogen in the system.

The losses are regulated by biotic processes, and studies of the roles of soil microorganisms and fauna in determining process rates is essential.

In view of the rapidly increasing additions of N fertilizers to modern

agricultural systems with concomitant concern for possible detrimental effects on the environment, it is important that we direct our attention to studies of the various factors regulating the biogeochemical N cycle to further our quantitative understanding of the fate of N in different cropping systems.

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Nitrogen relationships in intensively managed temperate grasslands

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Key words Ammonia volatilization Denitrification Grazed grassland Leaching N balance N losses Soil total N

Summary Most studies of N relationships in grassland have used cut swards. These have shown that for annual inputs of 200 to 400 kg N/ha from fertilizer or fixation, 55 to 80% of the N is recovered in harvested herbage. Generally, no more than 5 to 15% is lost through leaching and denitrification with most of the remaining N incorporated into soil organic matter. The relatively high efficiency of N use by cut swards reflects rapid uptake of N and the removal of a large part of the input in herbage. Inclusion of the grazing ruminant alters the efficiency of N use; only 5–20% of the input is recovered in meat or milk, and 75 to 90% of the N ingested is excreted, mainly as urea in urine. Application of N in urine ranges from 30–100 g/m^2. Too much N is voided for effective recovery by the sward whilst soils usually contain insufficient C to allow appreciable immobilization. The surfeit is lost. Hydrolysis of urea is usually complete within 24 h of urine deposition. For urine-treated pasture in New Zealand (NZ) losses by NH_3 volatilization of up to 66% of applied N are found during warm dry weather, with an average of 28% for a range of seasonal conditions. In the UK, the average rate of NH_3 loss from an intensively grazed ryegrass sward was 0.75 kg N/ha/day during a 6-month season. NH_4^+ remaining in the soil may be nitrified, nitrification being complete within 3 to 6 weeks. Although some NO_3^- is recovered by plants, a substantial portion is leached and/or denitrified. On average such losses were 42%, with only 30% of the added N recovered by plants in urine-treated pasture in NZ. In the UK annual leaching of 150 to 190 kg N/ha has been observed for grazed swards receiving 420 kg N/ha/yr. Low retention of N by grazing ruminants results in a breakdown of N relationships in intensively managed grasslands. The substantial losses through NH_3 volatilization, leaching and denitrification have serious agronomic, economic and environmental implications.

Introduction

N relationships in temperate grasslands are the subject of a large and increasing scientific and technical literature (*e.g.* Gandar[15], Lazenby[25]). This paper considers recent reserach, with emphasis on studies in intensively managed pastures yielding in excess of 10 t DM/ha, 60% or more of which is utilized annually. Our theme is the influence of biological processes on N relationships, particularly as affected by grazing ruminants.

Quantitative consideration requires that inputs and outputs must balance after allowance for any change in storage of N. For practical

purposes, storage in temperate grasslands is expressed through soil total N[3] so we consider this first.

Soil total N

Grassland soils frequently contain 5–15 tonnes N/ha in the root zone, most of which is present in organic matter[19] and arises from death and decay of roots and herbage. In soils resown after cropping total N usually increases at rates between 50 and 150 kg/ha/yr[6]. However, accumulation of N is finite, being asymptotic, with an equilibrium at the point where additions of organic residues are balanced against oxidation and losses. The time-course and factors affecting this process are poorly understood, but Garwood *et al.*[17] reported an apparent equilibrium in soil after 10 to 12 years of unchanged grazing management. In contrast, total N below permanent pasture usually remains roughly constant[22].

Input of C, rather than N, P or S, is the factor limiting organic matter and thereby N accumulation below intensively managed grassland. Several reports[2,6] have shown that accumulation is independent of N input so accumulation depends largely on herbage utilization. An extension of this concept is that, as management is intensified and utilization of herbage increased, insufficient C will be incorporated into soils to maintain existing levels of total N. Depletion of soil N at 50–200 kg/ha/yr has been observed under intensively managed pasture in NZ[2,8] mainly through loss of N from excreta as discussed below.

Inputs of N to grassland

The major inputs of N are from fertilizer and symbiotic N fixation. In many areas, particularly NW Europe and parts of N America, the amount of fertilizer N applied to grassland has increased substantially since the 1950's with rates as high as 400 kg N/ha/yr for intensive dairy farms in the UK and the Netherlands[14,36]. Inputs through symbiotic fixation vary appreciably with locality, but in swards managed to maintain a high legume content, estimates range from 74–280 kg N/ha/yr for lowland sites in the UK[7] and 85–342 kg/ha/yr for pastures in NZ[21]. Additional but smaller inputs (5–15 kg N/ha/yr) arise from N fixation by free-living microorganisms and from rain and dry deposition[33]. In grazed systems, up to 100 kg N/ha may be introduced through supplementary feeds[36].

Balance for N in cut swards

In numerous agronomic studies apparent recoveries in the range 55 to 75% have been observed for applications of fertilizer N up to 450 kg/ha/yr[27]. In grass-clover swards, apparent recoveries are generally lower, around 45%, partly because of reduced N fixation in the fertilized swards. After correction for N removed in herbage from an unfertilized ryegrass sward in NZ, Ball[2] found that about 80% of the N fixed by white clover in an unfertilized ryegrass-clover sward was recovered in herbage.

Recovery of N by a cut sward is determined by factors affecting its vigour, including temperature, available water, and frequency of defoliation. Lower recoveries, particularly at the higher rates of N input, reflect the increasing importance of N losses from the soil solution through leaching, denitrification, ammonia (NH_3) volatilization and immobilization.

Garwood *et al.*[18] reported an average annual loss through leaching of 8 kg N/ha from lysimeters under cut ryegrass swards receiving 250 kg N/ha, although half the amount applied during a summer drought was leached during the following winter[16]. Dodwell and Webster[11] reported leaching of 8–33 kg N/ha during winters following seasons in which 400 kg N/ha were applied; ^{15}N data showed that 60 to 70% of the total N loss was derived from fertilizer.

Recently denitrification losses from cut swards of between 5 and 9% of the N applied as ammonium nitrate at rates between 250 and 500 kg N/ha/yr were recorded for loam to clay-loam soils in SE England (annual rainfall approximately 650 mm) using the acetylene-inhibition technique[28,30]. Fig. 1 indicates that much of the loss occurred from the N applied immediately preceding wet periods in spring and summer, when the soil temperate was above 5 to 8°C and soil nitrate content in the top 20 cm exceeded 5 μg N/g (10–15 kg N/ha). Particularly high losses (15–30%) were observed following application of ammonium nitrate in spring to soil at field capacity. These were avoided by using equivalent amounts of N as ammonium sulphate or urea[29].

N may also be lost through NH_3 volatilization following application of fertilizer, particularly urea, although such loss is largely associated with grazed pasture, discussed below. Volatilization is enhanced by high temperatures, conditions favouring evaporation and neutral to alkaline pH[34].

With the exception of prolonged dry periods, there is little evidence to suggest that inorganic N accumulates in soil under cut swards. The persistent and dense root system allows rapid uptake of inorganic N, whether formed through mineralization or supplied as fertilizer.

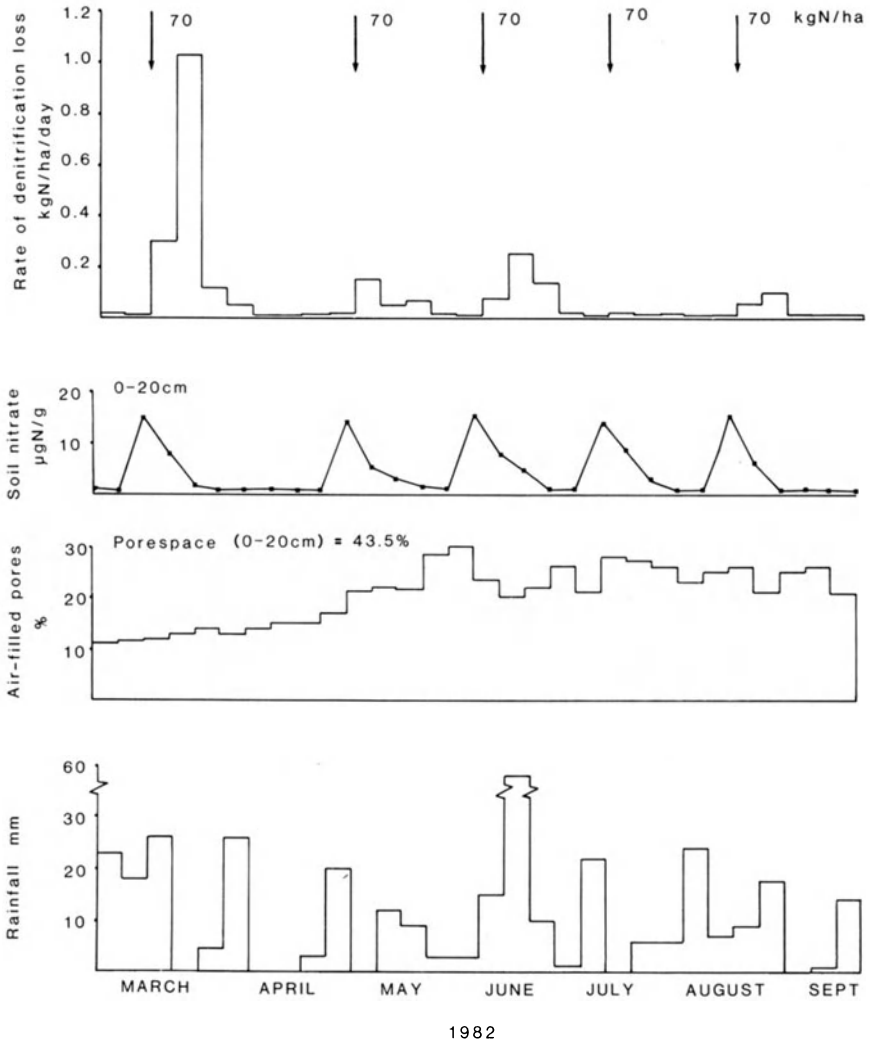

Fig. 1. Weekly mean denitrification rates measured using the acetylene-inhibition technique, soil nitrate contents, air-filled porespace and rainfall for a freely drained loam under a cut ryegrass sward receiving 350 kg N/ha/yr as ammonium nitrate in five equal applications (arrows).

Of the N that is not recovered in herbage or lost through leaching and denitrification, much accumulates in the soil organic matter and roots; up to 25% of the fertilizer N applied[12]. Kissel and Smith[24] using ^{15}N with Bermuda grass, showed that of a total residual organic N of 192 kg/ha, 70 were present in roots at the end of the season during which 520 kg N/ha were applied. At the end of the following season, only 27 kg fertilizer N/ha were associated with roots whilst that in soil organic matter had increased by 52 kg/ha. This suggests that root

death makes an important contribution to the retention of fertilizer N in soil.

Accumulation of N has to be weighed against that which becomes available through mineralization. Mineralization depends on soil type, climate and previous management, but is usually in the range 3–7.5% of total N in the top 15 cm each year, the higher values being associated with permanent swards[37].

Studies of N relationships in grassland involving cut swards have been useful in understanding plant response to N inputs and factors affecting recovery of fertilizer N. However, most grassland is grazed for part, if not all of each year. Pre-occupation with cut swards has obscured the vital question of the fate of the huge quantities of N removed in herbage, in particular that consumed by the grazing ruminant.

Impact of the ruminant on N relationships

At the simplest level a balance for N in grassland can be drawn up based on inputs and outputs at the farm gate. Van der Meer[36] has shown that for fourteen intensive dairy farms in the Netherlands only 16% of the total N input (525 kg N/ha/yr) could be accounted for in milk production and liveweight. At the highest levels of animal production from clover-based pastures in NZ, fat lamb, beef and dairy farming respectively yield outputs of only 25, 50 and 65 kg N/ha/yr[3]. In view of the magnitude of N inputs to intensively managed pasture, such data highlight the need to understand the ultimate fate of N.

In well-managed grazed pasture, ruminants consume 60 to 75% of the measured herbage and excrete 75% to 95% of the N ingested, mostly in the urine[37]. Applications of N in urine and dung patches range from about 30 to 100 g N/m^2. Urine-N, 70 to 90% of which is present as urea, is biologically labile. N in dung is present as organic compounds, mainly of bacterial origin, which are less readily hydrolysable than urea[19]: much of this N is probably incorporated into soil by the action of soil fauna. Although some NH_3 may be lost from dung[26], it is urine-affected areas that afford the greatest potential for N loss.

Studies of the fate of N in grazed systems follow two basic approaches. One involves the determination of the fate of N in urine-treated areas of pasture (*e.g.* Ball *et al.*[5]; Vallis *et al.*[35]) followed by integration into a model for a representative system[13]. Alternatively a whole system approach can be adopted in which the fate of N is determined for fields under representative management, made possible

Table 1. Recovery of mineral N (per cent of urine N applied)* in soil profiles (0–45 cm) following application of urine (Ball and Keeney[4])

Day	N30				N60			
	Urea	NH_4-N	NO_3-N	Total	Urea	NH_4-N	NO_3-N	Total
(a) Cool-moist								
1	3	51	1	55	5	64	1	70
6	trace	48	4	52	6	73	2	81
13	0	55	12	67	trace	44	6	50
20	0	37	19	56	0	34	12	46
31	0	11	33	44	0	24	27	51
45	0	2	31	33	0	9	37	46
88	0	0	9	9	0	1	20	21
(b) Warm-moist								
4	0	50	5	55	0	54	5	59
11	0	26	27	53	0	26	21	47
18	0	5	44	49	0	12	39	51
25	0	1	21	22	0	2	40	42
39	0	0	14	14	0	0	31	31
53	0	0	3	3	0	0	5	5
(c) Warm-dry								
3	5	31	1	37	3	68	1	72
10	1	38	9	48	1	55	13	69
17	trace	31	14	45	0	42	24	66
24	0	19	24	43	0	31	38	69
39	0	4	35	39	0	15	56	71
59	0	1	25	26	0	3	39	42
97	0	2	5	7	0	1	12	13

* Values corrected for control (NO)

Table 2. Fate of N, as affected by season, after application of urine to a ryegrass-white clover pasture in NZ. Values are relative to inputs of 30 and 60 g N/m^2 (Ball and Keeney[4])

	Seasonal conditions			
	Cool-moist	Warm-moist	Warm-dry	Mean
INPUT	100	100	100	100
RETENTION:				
By plants	53	27	10	30
In soil	0	0	0	0
LOSSES:				
Total	47	73	90	70
As NH_3	6	16	66	28

largely through improvements in aerometric techniques for measuring NH_3 loss[10].

Urine-N is rapidly lost as NH_3 following hydrolysis of urea, which is largely complete within 24 h of deposition (Table 1). In a detailed study of the fate of N in urine-treated areas of ryegrass-clover pasture

in NZ, the NH_3 loss, measured using a continuous-flow cover varied with season, being as high as 66% during warm dry conditions with an average of 28% for the three experiments conducted (Table 2). A similar pattern of loss was observed in an earlier, less detailed study in the UK[1]. In SE Queensland, Vallis *et al.*[35] observed that, depending on season, only 14 to 28% of N applied in urine (42.5 g/m^2) was lost from pasture receiving 374 kg N/ha/yr.

During and following the period of NH_3 loss, some NH_4^+ may be recovered by plants or immobilized in soil organic matter, but a significant portion is nitrified usually within three to six weeks of urine deposition (Table 1). Some of the NO_3^- may also be recovered by plants. However, two factors militate against plant recovery of inorganic N; first, the high salt concentration in urine often results in 'scorch' and sometimes death of plants[23] particularly during warm dry weather; second, the recovery of N even from split applications at rates in excess of 35 g/m^2 is notably poor[27]. The average apparent recovery of urine-N by pasture in NZ was only 30%, and as low as 10% during warm dry conditions (Table 2).

Leaching and denitrification are limited in cut swards through rapid removal of inorganic N from the soil solution[28]. However, in urine-affected areas of pasture soil nitrate contents frequently exceed 50–100 μg N/g[5] which, when soils are wet, support denitrification at rates >0.1 g N/m^2/day (Fig. 1). Some leaching seems inevitable if rainfall exceeds evapotranspiration. The unaccounted-for losses in Table 2 are largely attributable to a combination of leaching and denitrification. There was no accumulation of N in the soil, reflecting the fact that soils under productive pasture typically contain insufficient carbon to allow appreciable immobilization of excess inorganic N[4].

There are few data for N losses from whole grazed systems. Denmead *et al.*[9] reported NH_3 losses between 0.2 and 0.7 kg N/ha/day from lucerne grazed by sheep in Australia. Recent work in the UK (Fig. 2, Ryden[29]) has shown that rates of NH_3 loss frequently exceeded 1 kg N/ha/day during and following grazings in an intensively utilized system; yearling steers were rotationally grazed and the ryegrass sward received 420 kg N/ha/yr in seven equal applications, one before each regrowth interval. Losses were highest during and immediately after grazing and decreased during periods of rain (Fig. 2). Significantly, up to 24 days after grazing, NH_3 continued to be lost at rates between 0.2 and 0.5 kg N/ha/day. Between 15 and 30 kg NH_3-N/ha were lost during each 28-day period between consecutive grazings. No NH_3 loss was observed from cut swards receiving the same fertilizer N treatments.

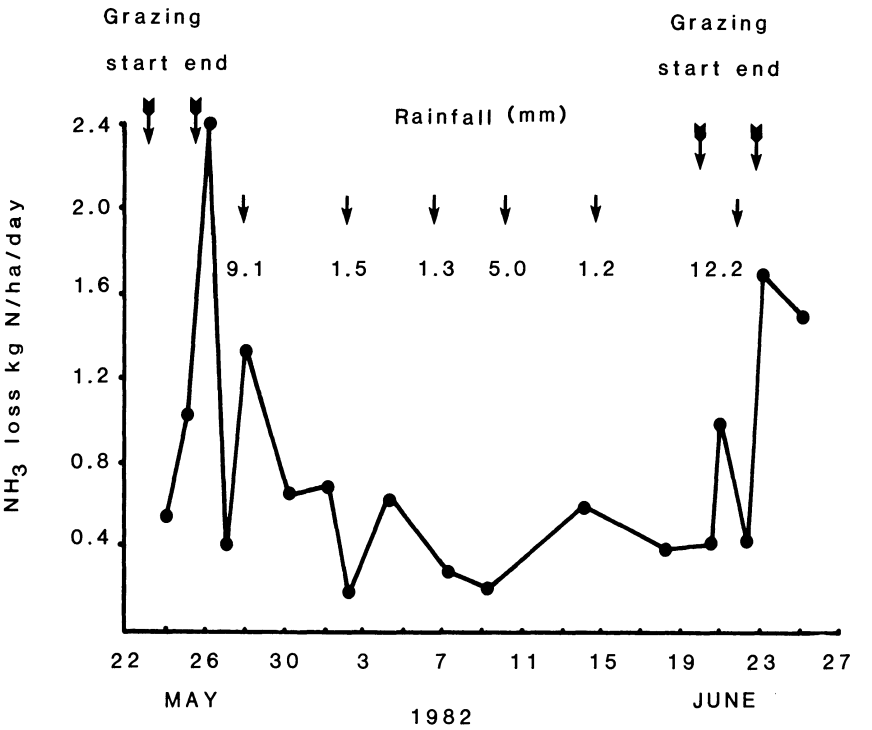

Fig. 2. Daily rates of NH_3 loss measured aerometrically during and after two-day grazings by yearling steers within a 28-day rotation: average stocking rate 11 head per ha. The sward received 420 kg fertilizer-N/ha/yr as ammonium nitrate in seven equal applications.

Table 3 indicates that mineral N in soil to a depth of 90 cm below the grazed ryegrass sward used in the UK studies of NH_3 loss was substantially greater at the end of the grazing season than that below the cut sward receiving the same amount of fertilizer N. The role of ruminants in the accumulation of mineral N is illustrated by the data for soil below "camped" and urine-affected areas of pasture (Table 3). While most was present as NO_3^-, these profiles also contained significant amounts of NH_4^+, some of which was present at 60–90 cm depth. Deep-core sampling into chalk below the grazed plots has confirmed that between 140 and 190 kg NO_3^--N/ha are leached each year. On the other hand, only 35 to 45 kg N/ha/yr was leached below the cut sward.

Other studies confirm that substantially more N is leached from soil below grazed swards than that indicated by studies in cut swards. Annual leaching loss from an intensively managed catchment in a high rainfall area of NW England increased from 30–40 to 50–80 kg N/ha as inputs increased from 188 to 290 kg N/ha/yr over a decade[20]. During the last five years of that study, an average of 30% of the N input left the catchment in stream flow. Below intensively managed pastures in

Table 3. Content of inorganic N in soil to a depth of 90 cm below cut and grazed ryegrass swards in UK receiving 420 kg N/ha/yr. The soils were sampled in November 1982

Soil sampled	Number of cores	NO_3-N content (kg/ha)	NH_4-N content (kg/ha)
At random across a grazed plot	39	160 (47–510)	10 (4–57)
Below the centre of urine affected areas	9	920 (200–1710)	258 (26–1090)
Below "camped" areas	6	400 (230–705)	36 (4–64)
At random across a cut plot	10	38 (22–74)	8 (6–11)

NZ annual leaching losses of 110–190 kg nitrate-N/ha have been observed in beef fattening systems[33]. Intensification of forage-livestock systems in Northern Ireland also accelerated leaching losses[31].

Conclusions

Cutting studies have been useful in defining plant responses to inputs of N, and factors affecting recovery of fertilizer N by grassland. However this paper illustrates the vital impact of grazing ruminants on the ultimate fate of N in intensively managed, temperate grasslands. In as much as grassland may be cut for forage conservation, the outstanding question regarding the fate of herbage N simply moves from the field to the feeding barn.

Benefits from the recycling of ingested nutrients by grazing animals are generally recognised. However, Ball and Keeney[4] challenged this view with respect to N due to the substantial losses induced by grazing ruminants. That view is supported by information presented in this paper.

Poor conversion of herbage N to products by ruminant animals results in a breakdown of N relationships in productive pastures. Pronounced concentration of excess dietary N into urine and dung patches promotes large losses, particularly from urine. Some soils below pasture in NZ are in negative N balance; combined losses, especially through NH_3 volatilization and leaching, exceed inputs. Continuing studies in the UK indicate that volatilization, leaching and denitrification together result in the loss of about 80% of N applied (420 kg/ha) to grazed perennial ryegrass. As most grassland is grazed, the conclusions to be drawn from studies of the ultimate fate of N in intensive pastural systems have serious agronomic, economic and environmental implications.

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Components of the nitrogen cycle measured for cropped and grassland soil-plant systems

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Key words Cereals Exchangeable ammonium Grassland Leaching loss Log-normal distribution Mineral N N balance Nitrate Steady-state

Summary Inputs and outputs to the N balance of a clay soil catchment (Evesham and Kingston series) under grassland and cereals at Wytham near Oxford were measured over 2 years. Soil mineral N ($NH_4 + NO_3$) was measured to 1 m depth at intervals of 2 to 8 weeks. The frequency distribution of these values was approximately log-normal and the geometric mean was used as an estimate of central tendency. Overall, soil mineral N tended to decrease during the study period, but marked fluctuations were observed in autumn (October–November) and early spring (February–March) in the grassland due to mineralization of soil organic N, and in the arable soil in April–May following the application of N fertilizer to the spring barley and winter wheat.

N lost by leaching, including a little surface runoff, was calculated from the NO_3 concentration of the catchment drainage and the volume of drainage. The estimate of N leached using concentrations unweighted for flow rate was only 14 per cent less than that based on flow-weighted concentrations. The differences in the uptake of N by cereals and grass between fields were explicable partly in terms of soil type and partly in terms of the timing and amounts of fertilizer added. The results are discussed in the context of steady-state equilibrium of N in the soil-plant system. However, an N balance could not be struck because N input due to mineralization, and N outputs due to gaseous losses and immobilization of N in the soil and root biomasses, were not measured and could not be accurately estimated.

Introduction

The conservation of soil N in agricultural systems is desirable because NO_3 loss in drainage and surface runoff is generally detrimental to the quality of potable water supplies, and its replacement in the form of N fertilizer is costly. Insight into the fate of N in the soil-plant system requires the study of gains and losses in soil N.

Theory

In the absence of significant biological N fixation, most of the transformations, gains and losses in soil N involve mineral N (NH_4 and NO_3-ions). Wild and Cameron[13] therefore wrote the equation quantifying the N balance for a prescribed area and time interval in the form:

$$N_p + N_f + N_m = N_{pl} + N_g + N_i + N_l + N_r + \Delta N \qquad (1)$$

where the subscripts are p = precipitation, f = fertilizer, m = mineralization of soil organic N, pl = plant uptake, g = gaseous losses, i = immobilization, l = leaching loss, r = runoff loss and Δ = change in soil mineral N. In the field situation, however, several of the terms in Eqn. (1) are difficult to measure accurately so various assumptions have been made to simplify the N balance[7]. A widely used assumption for a system under the same management for many years is that soil organic matter (and hence total soil N) reaches a steady-state value, and that N mineralization is balanced by organic N incorporated into crop residues (N_{cr}) and immobilization (N_i), *i.e.*

$$N_m = N_{cr} + N_i \tag{2}$$

Eqn. (1) then simplifies to

$$(N_p + N_f) - (N_{hp} + N_g + N_l + N_r) = \Delta N \tag{3}$$

where $N_{hp} = N_{pl} - N_{cr}$

Fried *et al.*[6] have simplified Eqn. (3) further by assuming that at steady state, inputs and outputs of N (L.H. terms in Eqn. 3) should balance so that ΔN, and not just the change in total soil N, must be zero. Thus, Eqn. (3) may be rearranged to give

$$N_l = N_{inputs} - (N_{hp} + N_g + N_r) \tag{4}$$

Fried *et al.*[6] argued that since the major N input was fertilizer, and physical losses such as N_g and N_r were usually small compared to N_{hp}, Eqn. (4) could be easily evaluated to predict the potential loss of N by leaching (N_l).

This concept is tested using data for arable and grassland soils measured over a 2 year period, from September 1979 to October 1981, at Wytham near Oxford.

Materials and methods

Site

The layout of the site is shown in Fig. 1 comprising three fields B, C and F (4.91 ha), under grass on the S side of a small stream and three adjacent fields, A, D and E (4.22 ha), on the N side under arable. The dominant grass species was *Lolium perenne* and the legume component was negligible. All the pasture and two of the arable fields had remained such for at least 50 years, but A had been ploughed from grass in 1971. The pasture was grazed on a month-on, month-off basis between May and October by year old beef cattle (5 per ha). Table 1 records details of cropping and additions of fertilizer (NH_4NO_3) for the six fields. In the year preceding the study A and D were winter wheat and E was spring barley. The land slopped at 7–9 per cent to the north east, and the soils graded from Evesham series (silty clay loam to clay) in the lower part (fields A, D in the arable and B, lower half of C in the grassland) to a calcareous variant of the Kingston series (sandy clay loam to clay loam) in the upper part (field E in the arable and upper C, F in the grassland). Tile and mole drainage

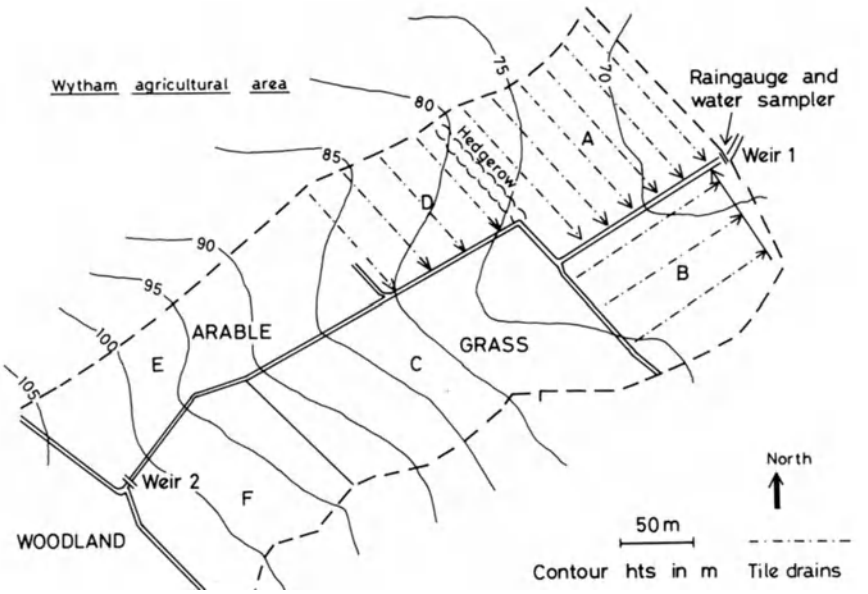


Fig. 1. Plan of grassland and arable catchment area of Wytham showing weirs and tile drains.

Table 1. Land use and fertilizer applications for the agricultural area of the Wytham catchment during 1979–80 and 1980–81 (from 1 September 1979)

Field	Area, ha	Land use	Sowing date	Harvest date	Appln. date	Amount	Fertilizer N, kg ha^{-1}	
							Annual rate of application	
							1979–80	1980–81
A	1.63	W. wheat	171079	210880	171079	23		
					140480	127	150	
		S. barley	070581	260881	120581	85		85
D	0.82	S. barley	130480	300880	130480	73	73	
		S. barley	070581	310881	120581	85		85
E	1.77	S. barley	130480	300880	130480	73	73	
			180481	010981	180481	85		85
B	1.15	Pasture			250380	83	83	
					270381	65		
					190681	40		
					100781	40		145
C	2.70	Pasture			250380	86		
					160580	43	129	
					080481	68		
					190681	43		111
F	1.01	Pasture			250380	86		
					110680	74		
					110780	66	226	
					270381	67		
					190581	66		
					100781	50		183

was installed in field A (1973) and in fields B and D (October 1980). Field drainage discharged into a small stream which flowed through the area from deciduous woodland higher upslope. A full description of the geology, soils and drainage is given by White *et al.*[12]. Soil pH (in 0.01*M* $CaCl_2$) was 7.5–7.7 in the arable and 7.3–7.7 in the grassland.

Rainfall measurements

Rainfall was measured in a "Rimco" recording raingauge with standard exposure near Weir 1 (Fig. 1). NO_3 and NH_4-N concentrations were measured in bulked samples at 1–2 weeks intervals by methods described below.

Soil sampling

2.5 cm dia. cores were taken to a depth of 1 m at 8 sites in the arable land and 12 sites in the grassland on 24 occasions between October 1979 and September 1981. The cores were subdivided into 4 depth intervals in the first year, and into 5 equal intervals subsequently. Larger cores (9 × 10 cm dia.) were taken less frequently for bulk density measurement.

Plant sampling

Grass growth was measured during 1981 by successive cuts to a height of 5 cm, from 1 m^2 quadrats within fenced enclosures excluding cattle. Six enclosures were sited at random in field C and three each in fields B and F. Sampling dates were 31 March, 6 May, 2 June, 30 June, 18 August, 24 September 1981 and 10 March 1982. Barley growth was measured by random sampling of shoot growth at 20 sites using a 30.5 × 30.5 cm quadrat. Sampling dates were 18 May, 10 June, 29 June, 14 July and 17 August 1981. The samples were dried in a forced draught at 80°C and weighed.

Drainage measurements

Stream flow was measured in V-notch weirs 1 and 2 (exit and entrance of the catchment area in Fig. 1), using stage recorders[9]. Discharge from the agricultural area (drainage plus any surface runoff) was calculated by difference. Water samples (0.5 dm^3) were collected from both weirs once weekly in 1979–80 and twice weekly in 1980–81, and analysed for NH_4, NO_2 and NO_3: NH_4 and NO_2 concentrations were < 1 g N m^{-3}. The daily flux of NO_3-N at each weir was calculated by two methods: (1) the mean daily NO_3 concentration was predicted from regressions of NO_3 concentration *vs* daily flow rate, derived by White *et al.*[12] (2) NO_3 concentration was taken as the measured value, or for days on which samples were not collected, as the mean of the closest measured values. Methods (1) and (2) were compared in 1979–80, but only method (2) was used in 1980–81.

Analyses

Water and rain samples were filtered through 0.4 μm "Nuclepore" membrane filters and analysed for NH_4, NO_2 and NO_3 by distillation in 1979–80[2] and by a "Technicon" auto-analyzer, series II, in 1980–81[5,8].

Soil samples (undried) were extracted in 2*M* KCl (1:10 ratio) and the filtered extracts analysed as above. Because NO_2 was negligible in nearly all samples, total mineral N was taken as NH_4-N + NO_3-N. Total N (soil or plant material) was determined by the Kjeldahl method[3]. Values in μg N g^{-1} soil were converted to kg N ha^{-1} using soil bulk densities.

Results

Soil mineral N

The NH_4 and NO_3 values varied greatly, with a tendency towards a skew distribution that suggested the population values were log-normally rather than normally distributed. Evidence of log-normally distributed data is provided by the fractile diagrams[11] in Fig. 2, compiled

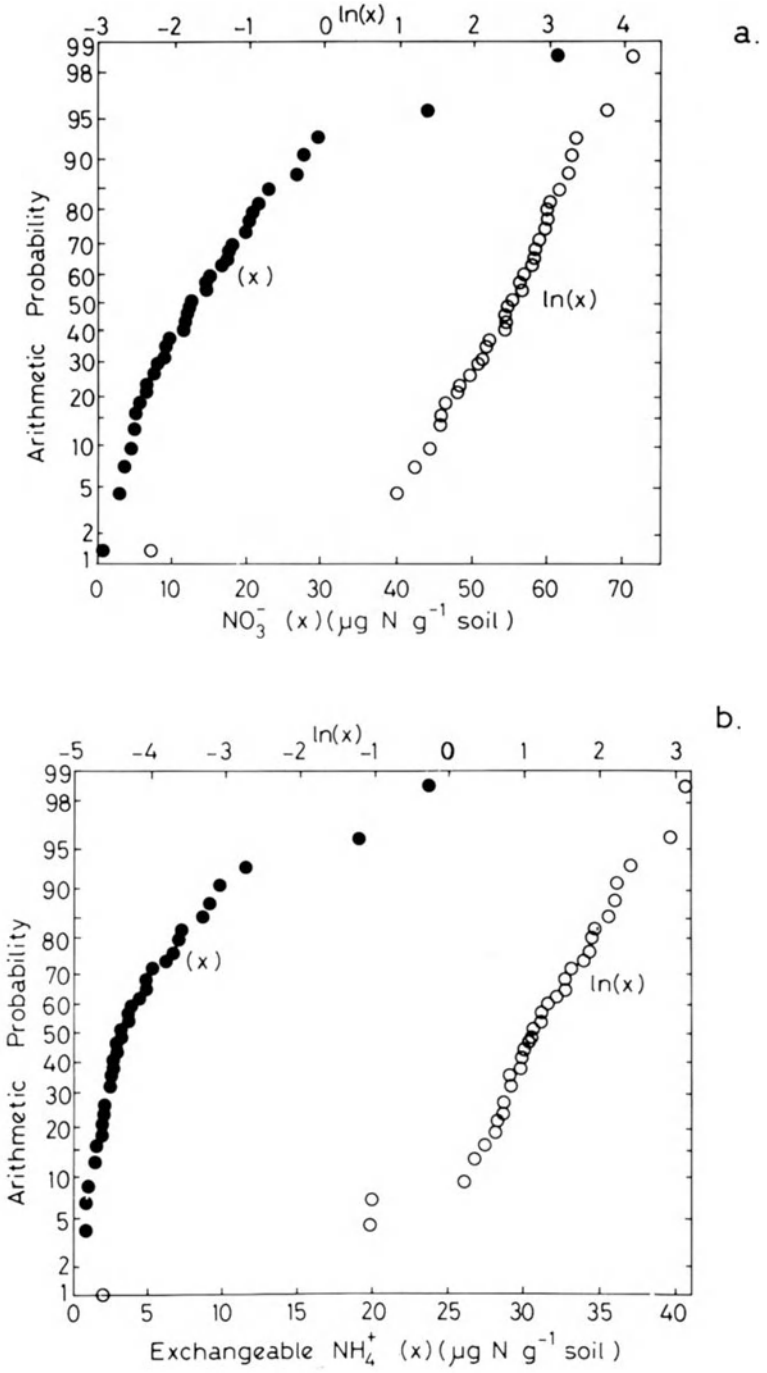
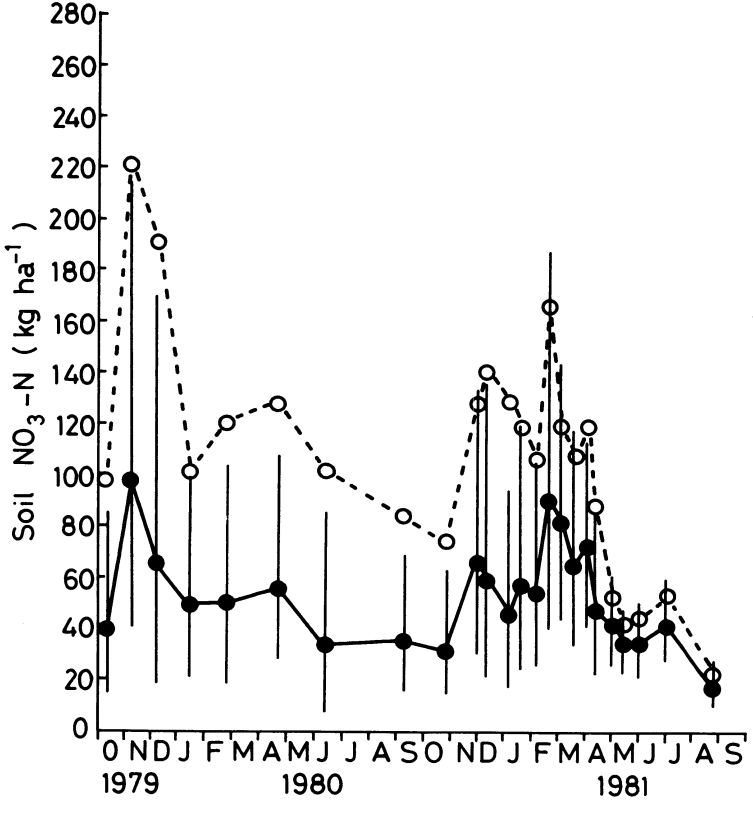
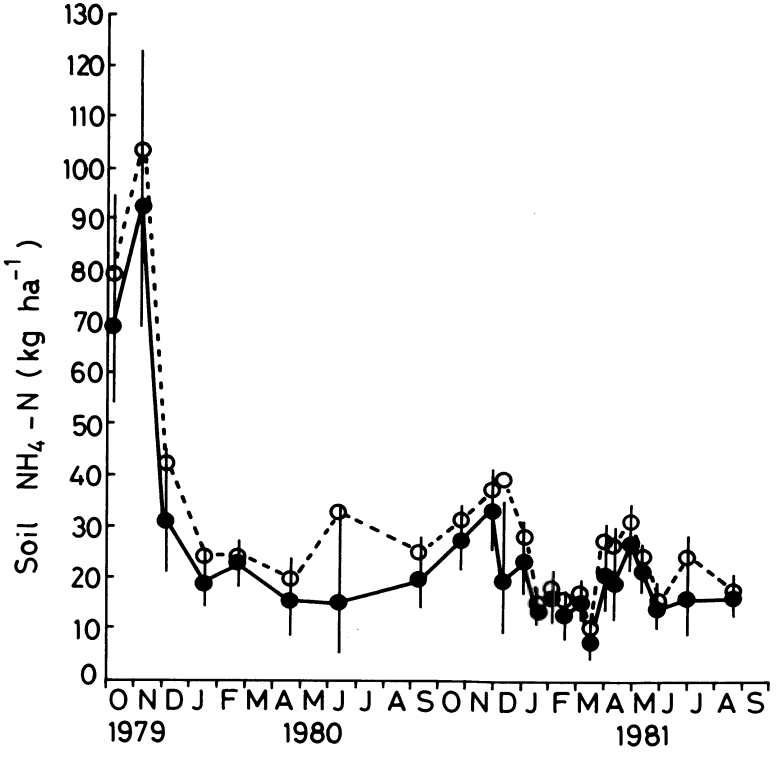


Fig. 2. Fractile diagram of exchangeable NH_4 and NO_3-N in 0–20 cm layer of grass field C on 26 March 1980.
(a) NO_3-N, (b) NH_4-N.

GRASSLAND


Soil NO_3-N (kg ha^{-1})
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
O N D J F M A M J J A S O N D J F M A M J J A S
1979
1980
1981


Soil NH_4-N (kg ha^{-1})
130
120
110
100
90
80
70
60
50
40
30
20
10
0
O N D J F M A M J J A S O N D J F M A M J J A S
1979
1980
1981

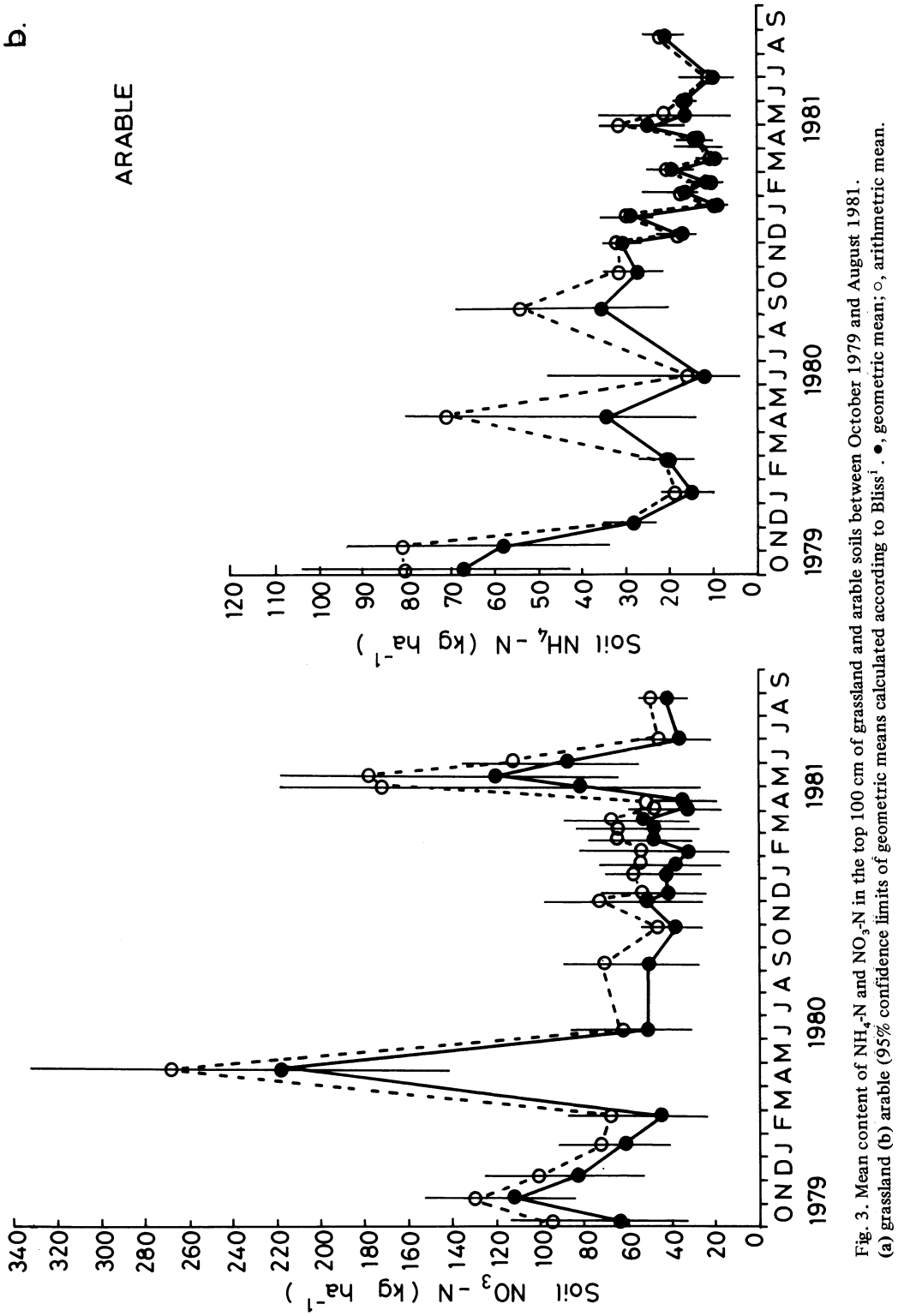


Fig. 3. Mean content of NH_4-N and NO_3-N in the top 100 cm of grassland and arable soils between October 1979 and August 1981. (a) grassland (b) arable (95% confidence limits of geometric means calculated according to Bliss[1]. ●, geometric mean; ○, arithmetic mean.

from 36 samples taken from grass field C at 0–20 cm on 26 March 1980. It was concluded that since the majority of samples belonged to log-normally distributed populations, the geometric mean was a better estimate of central tendency than the arithmetic mean.

The assumption of a log-normal rather than normal frequency distribution made a considerable difference to the estimates of mean NH_4 and NO_3 contents, especially for NO_3 in the grassland soil, as shown in Fig. 3a and b. The peaks in NH_4 and NO_3 contents in the arable soil in April–May were directly attributable to spring fertilization of the cereals (Table 1). However, there was no detectable effect of N fertilizer added to the grassland on soil mineral N levels. Changes in mineral N in the grassland seemed to be dominated by the mineralization of soil organic N in the autumn (1979 and 1980) and early spring (February–March 1981) when uptake of N by the grass was very slow.

Plant uptake of N

The cumulative uptake of harvested N by grass and barley (N_{hp}) in 1981 is shown for each field in Fig. 4a and b respectively. Quadratic regressions ($r^2 = 0.99$ for each field) were fitted to the grass data and used to calculate the weekly N uptake to the end of September. A final sampling on 10 March 1982 showed that uptake during the winter was small. The N uptake data for barley were best fitted by linear regressions ($r^2 = 0.93–1.00$) to mid-July 1981. After this time, N uptake appeared to decrease by harvest at the end of August, possibly due to a net translocation of N from shoots to roots, or to the loss of N in senescent tissues either through volatilization as NH_3 (Craswell and Martin[4]) or leaching by rain.

Leaching of N

The cumulative loss of N from the grassland and arable soils in drainage (N_l) and small amount of surface runoff (N_r) from 1 September 1979 is shown in Fig. 5. for 1979–80, the loss calculated by Method 1 (flow-weighted mean NO_3 concentrations) was 32.2 kg N ha^{-1}, and by Method 2 (unweighted mean concentrations) was 27.8 kg N ha^{-1}. For 1980–81, the loss was 48.7 kg N ha^{-1} (Method 2). The difference between the two years was mostly attributable to the leaching of 18.7 kg N ha^{-1} during the week commencing 18 May 1981, when 48 mm of rain fell. Fertilizer N had been applied to arable fields A and D on 12 May, and to field E on 18 April (Table 1).

N balance

For Eqn. (4) to be valid and of use to prdict N_l, the change in

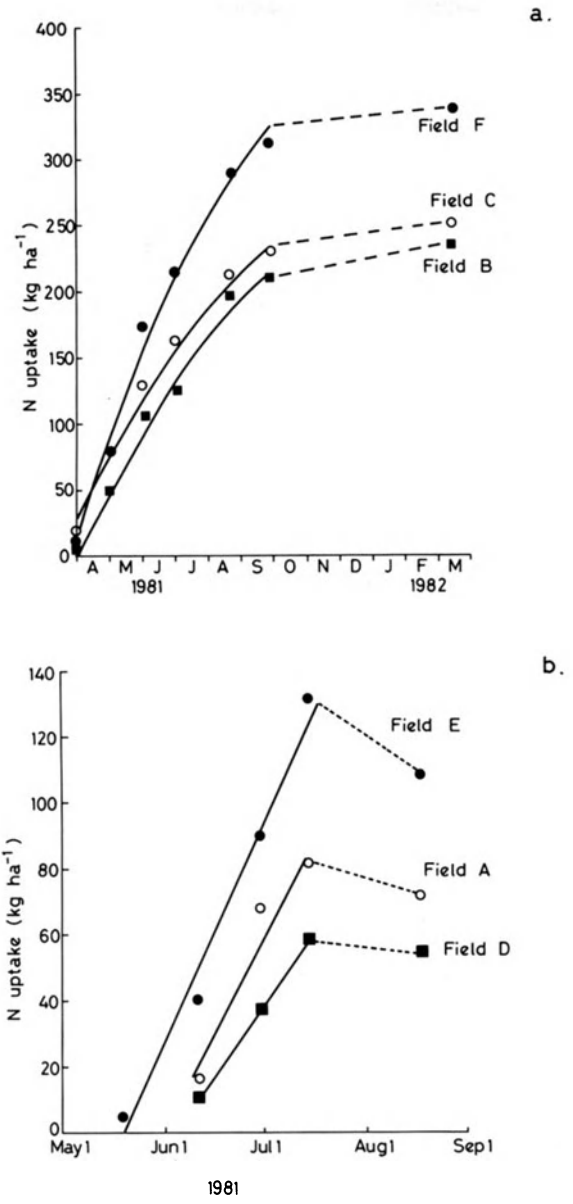


Fig. 4a. Cumulative uptake of N in harvested material in grass fields B, C and F during 1981–2.
Fig. 4b. Cumulative uptake of N in spring barley straw and grain in fields A, D and E during 1981.

soil mineral N (ΔN) over a given period should be zero. Although there are peaks and troughs in Fig. 3a and b, the overall trend in soil mineral N appears to be downwards. Moreover, the value of ΔN depends very much on the time of year from which measurements begin. This is illustrated in Fig. 6 where ΔN is calculated for successive 12 month

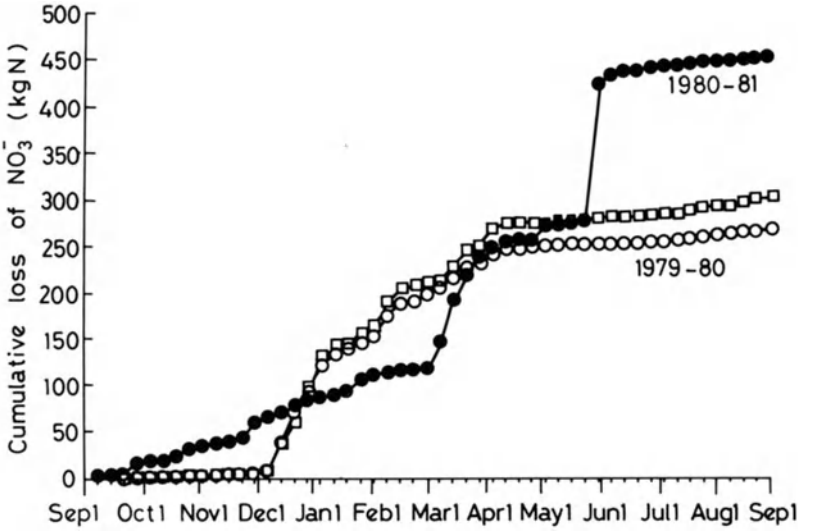


Fig. 5. Cumulative loss of nitrate from grassland and arable areas (9.13 ha) by drainage and runoff during 1979–80 and 1980–81 □ Method 1; ○, 6 Method 2.

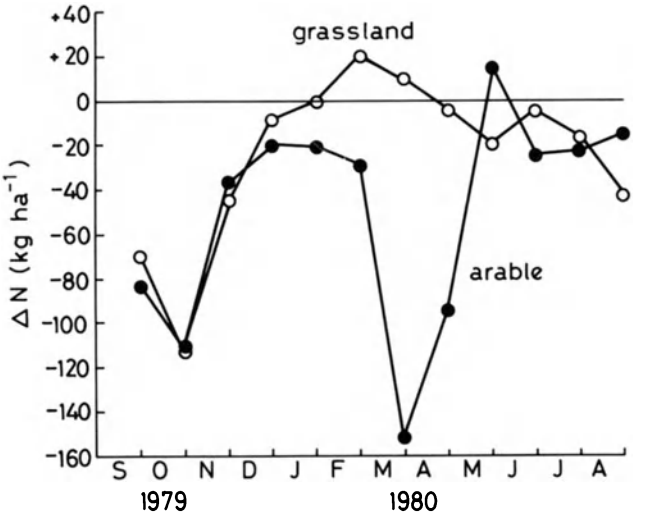


Fig. 6. Changes in mineral N (ΔN) in the top 100 cm of soil during successive 12 month periods starting on the first of each month between October 1979 and September 1980. ○ grassland; ● arable.

periods starting on the first day of each month from October 1979 to September 1980. The mean value of ΔN was − 59 and − 24 kg N ha^{-1} in the arable and grassland soils respectively.

Values of ΔN calculated for successive 4 week intervals in 1980–81 are given in Table 2. Substitution of ΔN, together with appropriate

Table 2. Input and output terms for N balance in the grass and arable area at Wytham during successive four weekly intervals in 1980–81. N in kg.ha^{-1}

Growth period	Four weeks starting	N_p	N_f	N_{hp}	$N_{l,r}$	ΔN	N_g' (= N_u)
Plant	290980	0.3	0	0	1.8	− 6.8	− 3.9
uptake	271080	0.3	0	0	3.2	+ 21.6	− 24.5
negligible	241180	0.6	0	0	2.2	− 13.1	+ 11.5
	221280	0.6	0	0	2.1	− 11.1	+ 9.6
	190181	0.3	0	0	2.3	+ 17.7	− 19.7
	160281	1.4	0	0	8.9	− 10.0	+ 2.5
Six month totals		3.5	0	0	20.5	− 1.7	− 24.5
Active	160381	1.1	35.7	13.4	7.3	− 4.8	+ 20.9
plant	130481	2.4	16.5	26.6	2.3	+ 30.2	− 40.2
uptake	110581	1.5	30.4	35.6	18.7	− 26.6	+ 4.3
	080681	0.2	17.7	46.0	1.0	− 15.7	− 13.4
	060781	0.8	10.5	21.6	0.3	− 4.2	− 6.4
	030881	0.8	0	8.5	0.4	− 2.6	− 5.6
Six month totals		6.8	110.8	151.7	30.0	− 23.7	− 40.4

values of N_p, N_f, N_{hp} and $N_{l,r}$ in Eqn. (3), rearranged in the form

$$(N_p + N_f) - (N_{hp} + N_{l,r}) - \Delta N = N_g \text{ (gaseous losses)} \quad (5)$$

provides "apparent" values of N_g (N_g' in Table 2). These are labelled "apparent" because, contrary to expectation, they are on average impossibly negative. This is probably because the assumption that Eqn. (1) could be simplified to Eqn. (3) does not hold, which suggests that to quantify the N balance for this catchment, the complete equation

$$(N_p + N_f + N_m) - (N_{hp} + N_{cr} + N_i + N_g + N_{l,r}) - \Delta N = 0 \quad (6)$$

is needed. Grouping the unknown terms as N_u, such that

$$N_u = N_{cr} + N_i + N_g - N_m \quad (7)$$

Eqn. (6) reduces to Eqn. (5), since

$$\text{apparent } N_g = N_g' = N_u \quad (8)$$

We conclude from the negative values of N_u (see Table 2) that throughout the year there was mineralization (N_m) of organic N in these soils (C:N ratios 7–12 in 0–20 cm layer) which substantially exceeded gaseous losses of N (N_g) and N immobilized in the soil and root biomasses (N_i and C_{cr}).

Discussion and conclusions

N uptake by grass and spring barley varied considerably between fields in 1981, partly due to differences in soil type and partly due to differences in the timing and amounts of N fertilizer applied. Comparison of grass fields B (Evesham series) and F (Kingston series) illustrates the interaction between soil type and N fertilizer. Field F received 38 kg N ha^{-1} more than field B but the total N uptake from F exceeded that from B by 188 kg ha^{-1}. In the case of barley, N uptake from field E (Kingston series) was greater than from fields D and A (Evesham series). Crop establishment in field D was particularly poor. It is likely that more of the fertilizer N was leached from fields A and D, where it was applied later than to E and was followed by very heavy rain during the next 14 d. Surface runoff from field E and from tile-drained fields A and D was minimal. NO_3 concentrations in tile drain flow ranged from 128–162 g N m^{-3} at peak discharge, suggesting that peak concentrations in rivers in spring[10] may be caused as much by leaching into tile drains as by surface runoff.

N leaching loss estimated from unweighted mean NO_3 concentrations derived from biweekly water samples was only 4.4 kg ha^{-1} (14 per cent) less than that estimated from flow-weighted concentrations. The difference was trivial compared to other components of the N balance and suggests that the extra effort required to obtain flow-weighted concentrations was not justified. Fried *et al.'s*[6] suggestion for predicting N losses by leaching could not be used in this experimental area because soil mineral N decreased with time. Measurements would need to be continued to establish whether this trend was long-term, or merely the downlimb of a harmonic-type fluctuation in mineral N which had a half-wavelength > 2 years.

Our measurements highlight the problems encountered in N balance studies due to spatial and temporal variations in soil mineral N, and the difficulty of validating any of the simplifying assumptions outlined above when one is working in a "real world" field situation. However, if the complete N balance equation (Eqn. 1) has to be used, measuring the contribution of each component with sufficient precision for a reliable N balance to be struck would require a prohibitively large and expensive sampling programme.

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The uptake of soil and fertilizer-nitrogen by barley growing under Scottish climatic conditions

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Key words Ammonium Barley Fertilizer Mineralization Nitrate Nitrogen Nitrogen-15

Summary Field experiments were carried out using ^{15}N-labelled calcium nitrate, to investigate the relative uptake by barley of fertilizer-N and soil-N. On imperfectly drained till soils uptake of soil-N increased with increasing rate of fertilizer, but remained constant on a brown sand, possibly due to more efficient root exploration in the latter soil. In four out of five seasons, late uptake of soil-derived N was a major feature, and uptake from ploughed soil as compared with uptake from direct-drilled soil was correlated with seasonal rainfall patterns. Significantly higher quantities of both fertilizer- and soil-derived N were taken up by winter barley than by spring barley, reflecting the longer growth period and higher dry matter yield from the former crop.

Introduction

Arable crops generally take up fertilizer N less efficiently than grass, resulting in substantial residues which may be either leached or lost by denitrification. Furthermore, fertilizer recommendations often diverge greatly from the optimum because of inability to predict the quantity released from soil organic matter. Optimisation of the use of N depends on better information on this soil contribution, but quantitative data applicable to Scottish environmental conditions are lacking. These conditions are characterised by lower ambient temperatures, lower moisture deficits and higher soil organic matter contents than occur in the more southerly parts of Britain. All these factors may be expected to have an effect on the extent or rate of mineralization.

The use of ^{15}N-labelled fertilizer, and the application of the principle of isotope dilution, make it possible to assess the relative contributions to the N uptake by plants derived from the soil N pool and from the fertilizer. Early studies on cereal crops in the field commonly used isotopic enrichments of 1 atom per cent excess ^{15}N or more (*e.g.* Bartholomew *et al.*[2]; IAEA[8]). However, Rennie and Fried[11] showed that lower enrichments of the order of 0.3 atom per cent excess ^{15}N could have been used without a significant increase in the error. Accordingly it was decided to use similarly labelled material in the present study, the intention being to investigate the effects of cultivations and soil texture on the relative contributions of soil-derived N and

Table 1. Soils on which experiments with ^{15}N-labelled fertilizers were located

Site	Soil series	Classification	Particle size analysis [a]				%C	%N	pH (0.01 *M* $CaCl_2$)
			Sand %	Silt[b] %	Clay %	Textural class			
Bush, Midlothian	Macmerry	Stagnogleyic brown earth[c]	49.5	35.2	15.2	L	3.1	0.22	6.6
	Winton	Cambic stagnogley[c]	47.5	35.3	17.4	L	2.2	0.16	6.5
Aberlady, East Lothian	Fraserburgh	Calcareous brown sand[d]	85	7	8	LS	1.4	0.14	7.8

[a] Topsoil
[b] 50–2 μm
[c] Till derived from carboniferous sediments
[d] Raised beach sediments

fertilizer-N to the total uptake by barley, which is the most important arable crop in Scotland both in terms of area planted and economic value.

Sites, materials and methods

The longest-running experiment (1978–82) was carried out at the Edinburgh School of Agriculture farm at Bush, Midlothian, on the ploughed and direct-drilled plots of a long-term continuous spring barley experiment[7]. This site included two soil series: Macmerry and Winton (Pidgeon[9]) classified (after Avery[1]) as a stagnogleyic brown earth and cambic stagnogley, respectively. In 1981–2 winter and spring barley were compared on a Fraserburgh series brown sand (raised beach) at Aberlady, East Lothian. Details of the soils are given in Table 1.

^{15}N-labelled calcium nitrate containing *ca* 0.35 atom per cent excess ^{15}N was prepared by mixing, in aqueous solution, calcium nitrate containing 5 atom per cent ^{15}N (BOC Prochem, London, UK) with a calculated quantity of the same salt but of normal isotopic composition, recrystallising, grinding to a powder and mixing again. Sub-samples were taken for isotopic analysis to confirm the actual enrichment achieved and its uniformity; results were satisfactory on all occasions.

A basal dressing of P and K fertilizer was applied to the seedbed in all experiments. Rates (kg ha^{-1} of P, K, respectively) were: 33, 62 annually at Bush; 11, 20 (winter barley) and 24, 46 (spring barley) at Aberlady. N fertilizer was applied separately in spring in all experiments. Labelled material was broadcast by hand over 2 m × 1.5 microplots located within larger main plots (at rates equivalent to 0–150 kg ha^{-1} at Bush in 1978 and at Aberlady, and 0–180 kg ha^{-1} at Bush in 1979 and 1980). The remainder of each main plot was similarly treated with unlabelled N fertilizer.

Plant samples (usually from 2 × 0.5 m lengths of row) were taken on 5 or 6 occasions during the growing season by cutting with shears within a few mm of ground level. Total N was determined in all samples by Kjeldahl digestion, and ^{15}N content was determined by mass spectrometry using the alkaline hypobromite method of Rittenberg[12]. Larger plant samples were taken from the main plots on each occasion to determine dry matter yield.

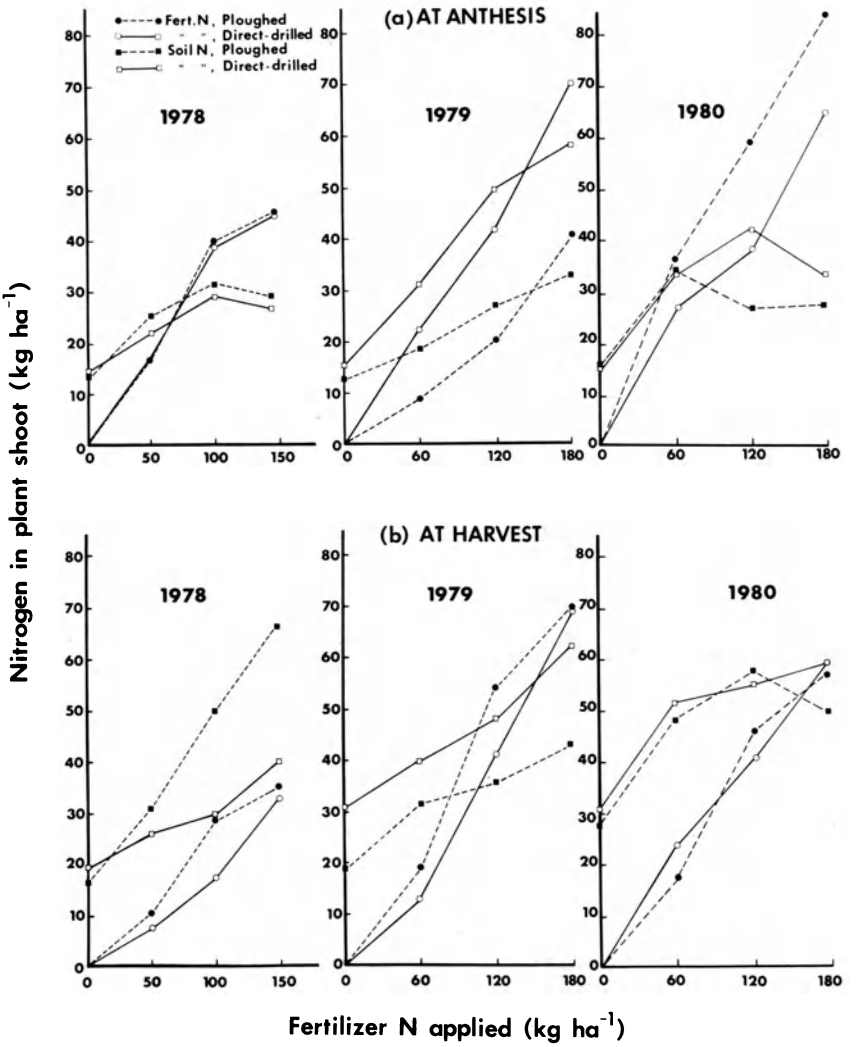

Fig. 1. Uptake of fertilizer and soil N in whole plant shoot as a function of fertilizer N applied: Bush Estate, 1978–1980.

Soil cores down to 40–50 cm depth were taken from a limited number of plots at Bush, using a 75 mm diameter lined corer. The cores were divided into 10 cm segments and stored at −15°C until analysed. Each segment (400–500 g) was extracted with 2 l of 1 *M* KCl, and NH_4^+ and NO_3^- were determined by continuous flow analysis (methods of Crooke and Simpson[5] and Henriksen and Selmer-Olsen[6] respectively). For the determination of the ^{15}N content of the NH_4^+ and NO_3^--N, it was desirable to have 1–2 mg of N_2 gas. This necessitated the use of the entire soil extract for many samples because the inorganic N content of the soil was only a few $\mu g\,g^{-1}$. The extracts were concentrated to *ca* 400 ml and steam-distilled[4]: the standard apparatus described by Bremner[4] was modified by replacing the 100 ml sample flask with a 1 l round-bottomed flask heated with an electrical heating mantle.

Soil particle size distribution, total C, total N and pH were all determined by standard methods[3].

Table 2. Percentage of N in plant shoot derived from soil at 3 N-fertilizer rates, ploughed and direct-drilled plots, Bush Estate

Year	Plant material	% of N derived from soil						SED[a]
		ploughed			direct drilled			
		N_1	N_2	N_3	N_1	N_2	N_3	
1978	Shoot at Anthesis	59.5	44.3	38.7	56.5	42.8	36.3	1.7
	Grain	74.3	62.3	60.1	79.3	64.1	58.7	3.5
	Straw	76.1	63.4	67.8	77.6	60.7	56.0	5.0
1979	Shoot at Anthesis	67.5	57.7	44.5	60.0	54.2	45.5	
	Grain	65.0	43.3	40.2	78.3	53.6	45.9	2.2
	Straw	63.6	40.3	39.4	72.3	54.8	46.0	2.8
1980	Shoot at Anthesis	48.2	31.5	24.9	55.2	53.8	33.5	
	Grain	74.0	56.4	49.0	69.8	56.2	51.2	3.5
	Straw	72.0	58.1	46.4	69.2	57.7	55.6	5.0

[a] Standard error of the difference

Results and discussion

Fig. 1 shows the uptake of soil and fertilizer N on the ploughed and direct-drilled plots at the Bush Estate site. Increasing the application of fertilizer N increased the uptake of fertilizer N by the plant and always lowered the proportion of soil N in the plant (Table 2). The application of fertilizer N also had an effect on the absolute uptake of soil-derived N by the plant. A comparison between unfertilized plots and the mean of the fertilized plots always showed a greater uptake of soil N on the fertilized plots. Also, at harvest in 1978 and 1979 and at anthesis in 1979, there was evidence of a positive linear response of soil N uptake to increasing applications of fertilizer N.

The tillage also influenced the uptake of soil N and the relative contribution of soil-derived N to total N uptake by the plants. In 1978, at harvest, the ploughed plots showed more uptake of soil N than the direct-drilled. Uptake of fertilizer N was also greater on the ploughed plots and the proportion of soil N in the plant at harvest did not show any tillage effect. Throughout the 1979 growing season the uptake of soil N was greater on the direct drilled than on the ploughed plots (Fig. 2). The proportion of soil N in the plant at harvest was also greater on the direct drilled plots (Table 2). However, the proportion of soil N in the plant at anthesis showed the opposite effect. It was higher than at harvest on the ploughed plots but lower than at harvest, particularly at the lowest rate of applied N, on the

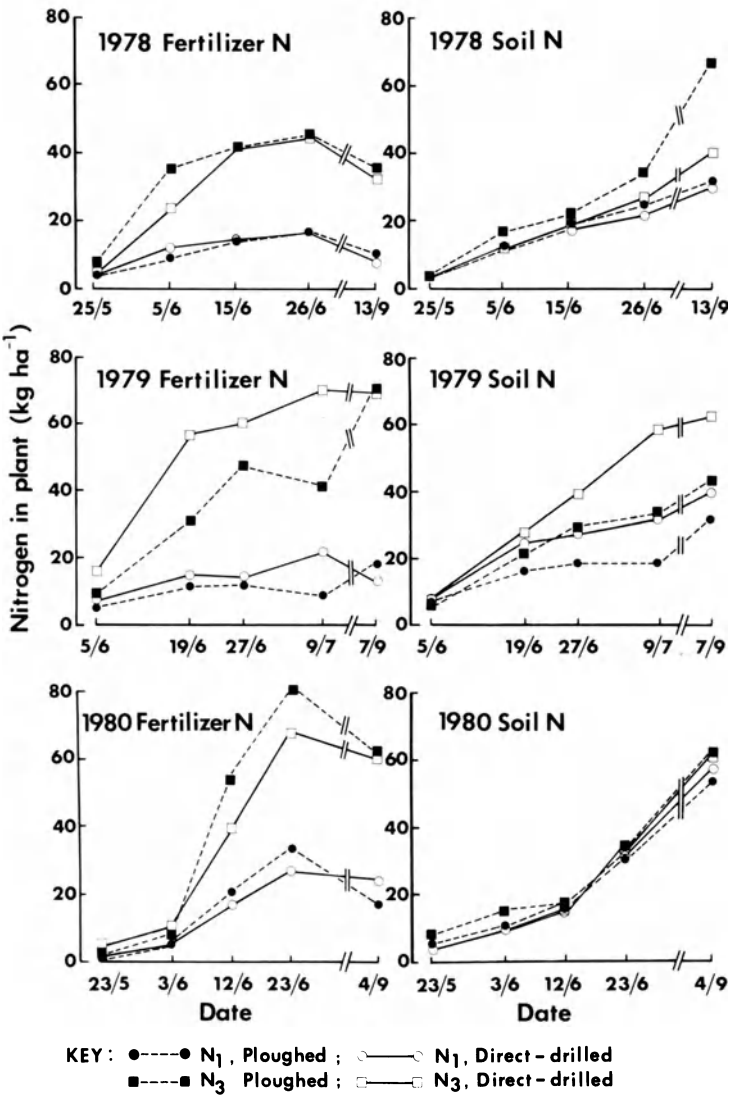
1978 Fertilizer N
1978 Soil N
1979 Fertilizer N
1979 Soil N
1980 Fertilizer N
1980 Soil N
Nitrogen in plant (kg ha⁻¹)
25/5 5/6 15/6 26/6 13/9
5/6 19/6 27/6 9/7 7/9
23/5 3/6 12/6 23/6 4/9
Date
KEY: ●----● N1, Ploughed ; ○——○ N1, Direct-drilled
■----■ N3 Ploughed ; □——□ N3, Direct-drilled

Fig. 2. Uptake of fertilizer and soil N during growth: Bush Estate, 1978–1980.

direct drilled plots. The proportion of soil N in the 1980 crop at anthesis was higher on the direct drilled than on the ploughed plots (Table 2), particularly at the higher rates of applied N. The proportion of soil N had risen by harvest but by much less on the direct drilled than on the ploughed plots.

In general, the proportion of soil N in the plant was higher at harvest than at anthesis, the exception being in 1979 on the ploughed plots (as discussed previously).

Table 3. Percentage of N in plant shoot derived from soil at 3 N-fertilizer rates, Macmerry and Winton Series, Bush Estate

Year	Plant material	% of N derived from soil						SED[a]
		Macmerry			Winton			
		N_1	N_2	N_3	N_1	N_2	N_3	
1978	Shoot at Anthesis	59.6	43.0	33.0	53.4	44.2	42.0	1.7
	Grain	78.9	69.8	64.5	74.6	61.6	54.3	3.7
	Straw	75.7	61.0	63.6	77.9	63.1	60.1	5.0
1979	Shoot at Anthesis	62.5	53.8	42.0	65.0	58.1	48.1	
	Grain	72.2	45.3	40.0	69.3	50.5	48.1	1.6
	Straw	58.5	37.5	43.5	67.7	56.2	54.7	2.9
1980	Shoot at Anthesis	55.6	39.7	28.5	47.8	45.6	30.0	
	Grain	75.1	62.0	56.3	68.7	50.5	43.9	3.8
	Straw	74.6	65.2	58.2	66.7	50.6	43.8	5.0

[a] Standard error of the difference

The proportion of soil N in the plant at harvest was also affected by the soil series upon which the plots were located. In 1978 and 1980 the proportion of soil N was greater on the Macmerry soil than on the Winton (Table 3). In these two years, greater increases in the proportion of soil N between anthesis and harvest on Macmerry gave rise to this difference at harvest. Generally the differences between soil series were small, although on Macmerry uptake of soil-N was usually higher than on Winton series, the exception being for the first cut in 1978.

At the Bush Estate site, the most important factor affecting the uptake of soil-derived N by the crop was the application of fertilizer N. In contrast, at the site near Aberlady in1981, the fertilizer N treatments had no effect on the uptake of soil N (Fig. 3). The contrast between unfertilized plots and the mean of the fertilized plots was not significant. Table 4 shows the percentages of plant N derived from soil. The proportion of soil N in the plant always decreased with increasing rate of applied N fertilizer. The application of fertilizer-N had a greater effect on the proportion of soil N in spring barley than in winter barley. Also, there was a higher proportion of soil N, at low rates of applied N, in spring than in winter barley. The reverse was seen at high N rates. Again there was a greater proportion of soil N in the grain at harvest than in the shoot at anthesis, for both crops. Data for 1982 will be reported elsewhere. At Bush Estate, the distribution of annual rainfall seemed to be an important factor

Table 4. Percentage of N in plant shoot derived from soil at 5 N-fertilizer rates between 30 and 150 kg ha^{-1}; winter and spring barley; Aberlady, 1981

Crop	Plant material	% of N derived from soil					SED[a]
		30	60	90	120	150	
Winter barley	Shoot at anthesis	75.3	58.8	43.2	39.2	37.3	5.1
	Grain	77.3	62.7	51.1	48.1	44.9	4.1
Spring barley	Shoot at anthesis	79.1	63.5	43.7	35.0	29.2	6.0
	Grain	82.7	63.9	59.9	46.0	38.9	5.0

[a] Standard error of the difference

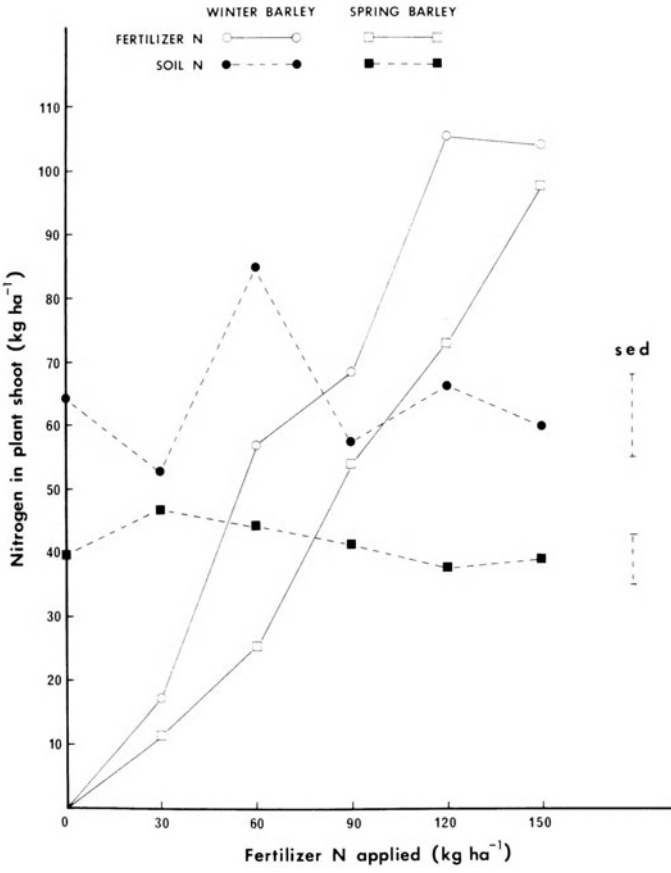

Fig. 3. Uptake of fertilizer and soil N in plant shoot at anthesis as a function of fertilizer N applied: Aberlady, 1981.

determining the difference between years. The total rainfall over the period March–May 1979 was much higher than for 1978 and for 1980 (275 mm compared with 164 and 115 mm respectively). This spring rainfall pattern in 1979 was followed by a lower total rainfall than in 1978 and 1980 for June and July (35.5 mm compared with 103 and 163 mm), accompanied by more sunshine from June onwards. In 1979 the soil water deficit remained relatively low until July as a result of the high rainfall in spring. In 1980, however, a large deficit had developed by May. This could explain the small amount of fertilizer-N in the crop in early June 1980 compared with that found in 1979. The higher rainfall during June in 1978 and 1980 could explain the June peaks in fertilizer-N uptake. There seems to be an interaction between the effects of tillage and the seasonal distribution of rainfall on the uptake of N by spring barley. In 1979 when the spring was relatively wet and the summer was dry compared with 1978 and 1980, direct drilled crops took up more N than ploughed, whereas in 1978 and 1980 direct-drilled crops took up less. Differences in topsoil bulk density and moisture content have been observed between the ploughed and direct-drilled soils at this site[10], as well as differences in root growth[7]. Under direct drilling the topsoil was more dense, wetter and had less roots per cm^3 than under ploughing.

Measurements at Bush Estate 8 weeks after sowing in 1979 showed that an overwhelming proportion of the $NH_4^+ + NO_3^-$-N in the profile was in the top 30 cm. The proportion of that N derived from soil ranged from less than 40 to 98%. (Detailed results will be published elsewhere).

The relationship seen at Bush Estate between the rate of applied fertilizer-N and soil-N uptake was absent from the brown sand at Aberlady. This was possibly due to more efficient root exploration at low rates of N in the latter soil, compared with the imperfectly/poorly drained soils at Bush, in which restricted root growth had been observed in earlier work[7,9].

Both sites showed an increased proportion of soil-N in the plant at harvest compared with anthesis. This suggests that mineralization late in the growing season may be a major factor influencing N supply under Scottish conditions of low spring temperatures and low moisture deficits.

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Leaching losses of nitrogen from a clay soil under grass and cereal crops in Finland

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Key words Barley Clay soil Fertilizer N Leaching loss Lysimeter Meadow fescue NPK fertilizer Timothy Winter wheat

Summary A 16-plot experimental field was established in 1975 on a clay soil in Jokioinen, Finland. The water discharge through tile drains was measured and its ammonium and nitrate N contents determined for each plot separately. The surface runoff was also measured and analysed. The annual runoff and the N leached from the surface of moderately fertilized (100 kg/ha/y N) cereal plots varied during 1976–1982 from 21 to 301 mm and from 2 to 7 kg/ha, respectively. The discharge of water and leaching of N through subdrains varied from 65 to 225 mm and from 1 to 38 kg/ha, respectively. The highest leaching was probably caused by a previous fallow. The annual N uptake by the crop varied between 41 and 122 kg/ha.

Of the fertilizer-N used for cereals, 20% of that applied in the autumn was lost, but only 1 to 4 per cent was lost when applied in the spring. There was much less N leaching from ley than from barley plots, although the former was given twice as much N. The rate of N fertilization had only a very slight effect on N leaching from both ley and barley plots.

The results were compared with those obtained in lysimeters filled with clay, silt, sand and peat soils. No definite conclusions can be drawn because the lysimeter experimental data are only for the first year.

Introduction

The leaching of nutrients is a harmful phenomenon which causes financial losses to agriculture and may cause environmental pollution. N, especially in the nitrate form, is particularly susceptible to leaching because it is not adsorbed by the soil. The leaching of N and other nutrients has been studied in Finland *e.g.* by Särkkä[9] and Kauppi[4] using measurements made on randomly selected drainage basins containing different kinds of land used for agriculture, forestry and other purposes.

In order to investigate more closely the effect of different cropping systems and fertilization as well as cultivation techniques on nutrient leaching, a special field was established at the Agricultural Research Centre in Jokioinen in 1975. It consists of plots which drain into separate drainage systems, the discharge of which can be measured and analysed.

There may be appreciable differences between soil types with respect to nutrient leaching and the effect of cultivation measured on it. In order to facilitate the comparison of soils under identical climatic conditions a lysimeter field was established in 1981.

In Finland over 50 per cent of the cultivated area is used to grow cereal crops. The area of winter cereals – rye and wheat – is smaller than that of spring-sown barley, oats and wheat. However, the leaching of nutrients, especially N, during the cultivation of winter cereals may be very important, because winter crops are partly fertilized at times when conditions are favourable for leaching. The time of application of the N fertilizer was therefore studied in the first two-year experiment on the field.

Grass for hay, silage or pasture is grown on 40 per cent of the cultivated land in Finland. The second experiment, which lasted three years, was therefore aimed at comparing cereal and grass growing at different fertilizer rates. In order to produce more variation in the water economy some plots were sprinkler irrigated.

Material and methods

Design of the experimental field

The soil on the experimental field contained 43–79 per cent clay fraction in its plough layer (0–20 cm) and was classified as silty or heavy clay according to the Finnish classification (Table 1). The clay content in the deeper layers (20–80 cm) was higher, varying between 62 and 94 per cent. The content of organic carbon, determined using the wet combustion method, varied from 2.2 to 3.6 per cent in the plough layer.

Table 1. Properties of the soils on the leaching experiment field and in the lysimeters, Jokioinen, Finland

	Depth, cm	Field (clay)	Lysimeter system			
			Clay	Silt	Sand	Peat
Org. C, %	0– 20	2.7	3.6	2.7	3.1	14.4
pH (water)	0– 20	5.9	5.8	6.0	5.9	5.6
	20– 40	6.3	5.8	6.5	6.0	4.7
	40– 80	6.9	6.6	7.0	6.2	4.8
	80–115	–	7.0	7.0	6.3	5.2
Particle-size fractions, %						
< 0.002 mm	0– 20	61 (43–79)	46	17	13	62
	20– 40	83 (62–93)	58	17	4	–
	40– 80	90 (73–94)	54	24	6	–
	80–115	–	54	16	9	–
0.002–0.02 mm	0– 20	16 (9–21)	15	62	8	8
	20– 40	8 (3– 6)	13	66	3	–
	40– 80	6 (2–14)	14	64	3	–
	80–115		18	51	4	–

The mean slope on the field was about 2 per cent, varying between 1 and 4 per cent.

Drainage pipes (ceramic) had been laid in the experimental field in 1962. The pipes had an inside diameter of 40 mm and were laid parallel, 16.5 m apart and at a depth of about 1 m.

In autumn 1975, the drains were cut into 33 m lengths and connected to plastic pipes, forming 16 plots each with two drains 33 m in length. The drain water from each plot was conducted to an observation building and its volume measured. A representative sample was taken from each measured lot of water.

The surface water collected in four wells situated at the lower edge of the field was also measured and analysed.

Experiment on application times of N fertilizer

In autumn 1976, an experiment was established in order to study the role of the application time of N fertilizer for winter wheat. There were four treatments: (1) no N fertilizer and (2) 100 kg/ha N as calcium ammonium nitrate given at sowing in September, (3) the same applied to frozen soil in November or (4) in May at the beginning of the growing season. The treatments were arranged on the field according to a Latin square.

The winter wheat was sown on September 5. An adequate amount of P and K was applied to the entire field. This fertilizer and the calcium ammonium nitrate for treatment (2) were placed 8 cm below the soil surface. The fertilizer for the other treatments applied on Nov. 26 and May 10 was broadcast. In order to prevent fertilizer leaching to the drains of neighbouring plots only a central strip covering 65 per cent of the plot area was treated. The winter wheat was harvested on September 5 at full maturity. The straw yield was also determined, and the straw ploughed into the soil.

The experiment was repeated the following year. The winter wheat was sown and treatment (2) given on September 20. The other N application times were November 29 and May 8. Unfortunately the wheat did not survive the winter. Therefore spring barley was sown on the field on May 15 1978. It was harvested on August 30. The straw was ploughed in.

In 1979 spring barley was grown on the field. It, too, was harvested in order to determine the residual effect of the treatments.

Experiment on fertilizer rate for cereals and grass ley

In 1980–82 a factorial experiment was performed on the field. It consisted of three factors with two levels each:

factor 1, crop: a. barley, b. ley (timothy + meadow fescue)

factor 2, NPK fertilizer rate: a. low, b. high

factor 3, irrigation: a. not irrigated, b. irrigated (30 mm in June)

Each treatment had two replicates. The annual amounts of N, P and K applied were (kg/ha):

	low rate			high rate		
	N	P	K	N	P	K
barley	50	21	40	100	42	80
ley	100	42	80	200	84	160

The barley was sown and fertilized simultaneously with a combination drill which placed the fertilizer between the seed rows at a depth of c. 8 m. The dates of sowing in consecutive years were May 7, 13 and 10. The barley was harvested at full maturity on August 14, September 9 and August 26, respectively. The straw yield was determined, and the straw ploughed in every autumn. The ley had been seeded in 1979 with timothy (*Phleum pratense*) and meadow fescue (*Festuca pratense*). The grass was cut twice a year: first at the end of June, and secondly in August. The annual fertilizer dose was split into two equal portions, one broadcast in May at the beginning of the growing season, and the other immediately after the first cutting.

Lysimeter field

The lysimeter system is similar to the system in Askov, Denmark (Kjellerup and Dam Kofoed 1983). It consists of 96 containers, 0.9 m in diameter and 1.7 m deep made of fibreglass reinforced plastics. There is a layer of pure sand (particle-size composition: 67 per cent 0.06–0.2 mm, 33 per cent 0.2–0.6 mm) on the bottom of each container. The lysimeters are drained through plastic tubes, and the drainage water is weighed and sampled for analysis. There are four soils in the system, 24 lysimeters filled with each soil. Two lysimeters of each soil were taken as monoliths using the method reported by Belford (1979): the others were

filled with loose soil in the natural depth sequence 115–80 cm, 80–40 cm, 40–20 cm and 20–0 cm. Some properties of the soils are given in Table 1.

On June 4, 1982, spring barley was sown in all lysimeters. A dressing of 100 kg/ha N, 20 kg/ha P and 40 kg/ha K was applied to the clay, silt and sand lysimeters as a compound fertilizer. The peak lysimeters received 10 kg/ha N, 30 kg/ha P and 60 kg/ha K as a compound fertilizer. In order to ensure good growth the lysimeters were irrigated during the summer with 95 mm water. The barley was harvested on September 29.

Analytical methods

The water samples were analysed for NH_4 and NO_3-N. In 1976–81 this was done with ion-selective electrodes manufactured by Orion Research Co., USA. Later on an AKEA auto-analyzer manufactured by Datex, Finland was employed. The ammonia was determined colorimetrically ($\lambda = 630$ mm) using the phenol-hypochlorite method with heating to 60°C. The nitrate was reduced to nitrite with a copper-cadmium column and determined colorimetrically ($\lambda = 520$ mm) using sulphanilamide and N-l-naphthylkethylenediamine dihydrochloride reagents. In samples of plant material, the total N was determined using a standard distillation as ammonia after Kjeldahl digestion.

Results and discussion

The form of N in the water

Only NH_4 and NO_3-N were determined in the water samples. Because the soil on the experimental field has a moderate humus content some soluble organic N is probably leached, too. On the other hand, in these trials only mineral fertilizers were used and their effects studied. The role of organic N leached may thus be considered less important. The content of NH_4 in all samples was so much smaller than the NO_3 content that it could be neglected.

The annual variation in N leaching

In 1976, the field was in fallow, and thereafter cereals have always been grown on some part of it. Barley fertilized with 100 kg/ha N has always been grown on some of the plots since 1978. Table 2 shows the precipitation measured at Jokioinen 1 km from the field, as well as surface and subdrain discharges and the amounts of nitrate nitrogen leached from the above-mentioned plots.

As expected, the surface and subdrain discharges are correlated with the precipitation. However, some exceptions also occur. For example, the precipitations for 1980 and 1982 were almost the same but the latter gave much higher surface and drain discharges. The main reason might be the warm and rainy December 1982, during which the soil was not frozen as usual. An unexpectedly high surface runoff in 1981 was clearly caused by the unusually rainy summer.

The leaching of N in 1976–77, mostly during the autumn and spring, was very much higher than since. The main reason was probably the

Table 2. Leaching of NO_3-N and N uptakes in cereal crops fertilized with 100 kg/ha N, Jokioinen, Finland

	1976	1977	1978	1979	1980	1981	1982	Means
	Winter wheat		*Spring barley*					
Precipitation, mm	373	630	484	657	645	709	644	592
Surface water, mm	21	96	61	89	110	301	183	123
NO_3-N, kg/ha	4	5	3	2	2	7	5	4
Drainage water, mm	65	225	84	162	136	156	188	145
NO_3-N, kg/ha	26	38	1	3	5	10	5	13
Total leaching								
NO_3-N, kg/ha	30	43	4	5	7	17	10	17
Uptake by crop								
N, kg/ha	–	122	75	56	73	41	62	72

fallow in 1976, during which the soil N was effectively mobilized but taken up only sparingly by the following winter wheat.

N leaching naturally depends on the rate of surface runoff and drain discharge. However, not even in the exceptionally wet year of 1981 was as much N leached as in 1976–77 after fallow. Kauppi[5] has estimated the N leaching from cultivated land to be approximately 12 kg/ha/y. This figure fits within the range of the present results, but is probably above the usual level under the prevailing conditions. Greater leaching was observed in Denmark by Rasmussen[8] and Frimodt Pedersen[3], and also by Kjellerup and Dam Kofoed[7]. The Danish soils contained less clay fraction, and probably had shorter frozen periods, which explains at least part of the difference.

There was no close correlation between N uptake by the crop and N leaching. In 1977 the winter wheat took up much more N than the barley grown thereafter, and there was more leaching, too, than usual. One reason for both might be the preceding fallow. In the wet year 1981 the crop grew poorly and took up less N than usual, which facilitated high leaching.

Application time of N fertilizer

The winter wheat yield in the first experimental year, without fertilizer N, was high, obviously because of the preceding fallow (Table 3). Even so, quite a large yield increase was obtained by N fertilization. Yield increase did not depend on the application time, but because of the raised N content in the grain the N uptake was higher when N was given in May than when given in the preceding autumn. Much N leached through the tile drains, without any applied fertilizer, but even more escaped when N was applied, particularly in the previous autumn. However, the increase was quite small after application in May. The differences in N uptake are well explained by variations in the subdrain

Table 3. Grain and straw yields; N removal in crops and by leaching

		Without N fertilizer	Increase due to 100 kg/ha N applied in		
			Sept.	late Nov.	early May
1976–1977 (winter wheat)					
1. Grain yield	kg/ha D.M.	2550	+ 880	+ 890	+ 830
2. Straw yield	" "	2750	+ 1110	+ 1050	+ 1170
3. N uptake	"	70	+ 30	+ 34	+ 52
4. NO_3-N leaching	"	54	+ 21	+ 19	+ 4
5. N removal	" (3 + 4)	124	+ 51	+ 53	+ 56
1977–1978 (barley after winter wheat failed)					
1. Grain yield	kg/ha D.M.	680	+ 600	+ 1550	+ 1840
2. Straw yield	" "	670	+ 560	+ 1540	+ 1870
3. N uptake	"	19	+ 13	+ 40	+ 56
4. NO_3-N leaching	"	6	+ 19	+ 0	+ 1
5. N removal	" (3 + 4)	25	+ 32	+ 40	+ 57
Residual effect 1978–1979 (barley)					
1. Grain yield	kg/ha D.M.	2920	+ 120	+ 20	+ 190
2. Straw yield	" "	1420	+ 180	+ 40	+ 70
3. N uptake	"	57	− 1	− 2	+ 0
4. NO_3-N leaching	"	3	+ 0	+ 0	+ 1
5. N removal	" (3 + 4)	60	− 1	− 2	+ 1

leaching since the sums of uptake N and leached N for the three different application times were nearly equal *i.e.* 51–56 kg/ha.

The barley sown in 1978, after the winter wheat failed, grew very poorly without N fertilization but responded well to the 100 kg/ha N given at various times. The effect on both grain and straw yield was very dependent on the application time; in May just before sowing the barley gave the most, and in the previous September the least. N uptake varied in the same way. However, the leaching through subdrains did not explain the differences in uptake. The lack of residual effects in the following year – 1979 – (Table 3) indicate substantial losses of fertilizer N from the soil other than by leaching, particularly when N was applied in autumn.

Fertilizer rate for cereals and ley

In the 1980–82 experiment, every second plot was irrigated with 30 mm of ground water in June. Irrigation increased the dry matter grain yield of barley by an average of 70 kg/ha, which was statistically insignificant. Similarly, neither the average grass dry matter yield nor the N uptake increased significantly ($P = 0.05$). The irrigation caused a small, nonsignificant increase in N leaching through the subdrains, too, but only at the higher NPK level: it averaged 2 kg/ha for both barley and ley.

The annual yields varied widely because of the very poor growth in 1981. In spite of this the treatment effects were similar and thus only the means over the entire experimental period are given in Table 4. The yield levels obtained agree quite well with those in practical Finnish agriculture but are clearly less than usual in field experiments. The responses of barley and ley to N fertilization were as expected. The apparent recovery of fertilizer N between the rates 50 and 100 kg/ha/y was 42 per cent for barley and 56 per cent for grass between the rates 100 and 200 kg/ha/y.

N leaching through the subdrains was much larger under cereal crop than under ley, agreeing well with the results reported by Bolton *et al.*[2] and Kjellerup and Da, Kofoed[7]. The more effective uptake by the latter explains part of the difference. Another factor is that the ley still takes up N in late summer when the uptake by barley has already ceased.

Increasing N fertilization increased leaching losses very little, so other losses must have been substantial according to the poor apparent recovery. The result agrees well with those of Kjellerup and Dam Kofoed[6]. In their field experiment performed on a clearly lighter soil than the present one there was no marked increase in N leaching when the N fertilization of barley was raised from 110 to 165 kg/ha. Similar results have been obtained by Kjellerup and Dam Kofoed[7] in lysimeters in most years, although the conditions were much more favourable for leaching, including relatively light soil and very high water discharge.

The lysimeter experiment

The results obtained in the 1982 field experiment and those in the lysimeters are compared in Table 5. Because the lysimeters were covered until April 30, the leaching is calculated only from that date to the end of the year.

The grain and straw yields on the field and in the clay lysimeters were not markedly different. However, the N uptake in the lysimeters was much higher than on the field, which indicates a higher N content in the plant material. The water discharge from the clay lysimeters was

Table 4. Mean yields of barley and grass, N uptakes in crops and losses by leaching during 1980–82

		Barley		Grass ley	
N applied kg/ha/y		50	100	100	200
Grain yield,	kg/ha/y D.M.	2060	3050	–	–
Straw yield,	"	1490	1940	–	–
Grass yield,	"	–	–	3850	6060
N uptake,	"	37	59	56	112
NO_3-N leaching,	"	6.8	6.4	1.4	3.0

Table 5. Mean yields of barley grain/straw, N uptakes and leaching losses from field plots and lysimeters, 1982

			Lysimeters			
	Field (clay)		Clay	Silt	Sand	Peat
N application, kg/ha	100		100	100	100	10
Grain yield, kg/ha D.M.	3350		3940	3990	4280	3880
Straw yield, kg/ha D.M.	1610		2540	2690	3400	2760
N uptake, kg/ha	62		106	94	141	90
Surface subdrain						
Discharge, mm (May–Dec.)	84	94	230	138	173	243
NO_3-N leaching, kg/ha	2.1	2.9	3.6	4.2	4.2	1.6

greater than the total discharge from the field surface and drains, even though no surface runoff can occur in the lysimeters.

The differences that can be seen between soils in Table 5 do not permit any final conclusions because the newly filled lysimeters had probably not yet reached a steady state. The leaching in all soils was less than that found by Kjellerup and Dam Kofoed[7] in loam lysimeters, even if the short observation period and smaller water discharge is taken into consideration.

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Tillage effects on nitrogen uptake by maize from fine textured soils in the northwestern Corn Belt, USA

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Key words Maize Nitrogen Nitrogen uptake Tillage *Zea mays* L.

Summary Nitrogen (N) accumulation data from a replicated field study were fitted to a tanh (time) function and the derivate obtained to determine relative maximum rates of accumulation by maize. Both positive and negative effects of tillage on N accumulation rates were observed. Most of the N accumulation occurred over a 30-day period and time of N accumulation was not affected by tillage. Tilled profiles tend to contain greater NO_3-N, greater aeration, and lower moisture contents than untilled profiles, and these characteristics interact to affect plant N accumulation.

Introduction

A general trend toward use of less tillage, especially in maize (*Zea mays* L.) production has been underway in the United States over the past 30 years. Several factors including tillage costs and concern over accelerated soil erosion associated with many practices have encouraged less intensive tillage systems in crop production. Still, acceptance of less intensive tillage systems in the northwestern Corn Belt has been slower than in other areas.

Changes in tillage methods cause changes in soil physical and chemical characteristics which affect crop growth. Untilled soils have larger bulk densities, B_D, and volumetric water contents, θ, in the surface horizon at planting time. The combination of increased B_D and θ result in less aerated profiles suggesting that denitrification affects NO_3-N concentrations in untilled soil profiles. Larger microbial denitrifier populations and smaller NO_3-N concentrations are found in untilled soils[5,6,13].

While maize uses both ammonium N (NH_4-N) and NO_3-N[16] some workers strongly maintain NO_3-N is the main uptake form[8]. Maize grown without tillage should show greater response to N fertilizer in general and NO_3-N in particular if the importance of NO_3-N is accepted. Without tillage, Bandel *et al.*[2] obtained greater yields with NH_4NO_3 than with urea fertilizers. Also, Thomas *et al.*[13] and Bandel *et al.*[1] found that yields were unaffected by tillage when N fertilizer rates were optimum for tilled soils. But, when N fertilizer rates

were less than optimum for tilled soils, yields were greater with tillage.

The contribution of nutrients from previous crop residues is sometimes variable and depends in part on relative resistance to microbial decay. A field study was started in 1980 to estimate tillage effects on fertilizer and residue N use efficiency on finer textured soils in the northwestern Corn Belt.

Site descriptions and methods

Three tillage systems were used: fall moldboard plow-spring disk (FP), fall chisel-spring disk (FC) and no-till (NT) before planting. All plots were cultivated twice after plant emergence. Each treatment was replicated three times on Nicollet cl and four times on Wentworth sicl. Plots measured 9.1 × 18.3 m and maize was planted at a rate of 60,000 seeds/ha in 75-cm row spacings. All plots received about 30 kg P/ha broadcast and either NH_4NO_3 or urea before all tillage. Mixed fertilizer (N, P, K) was band applied in the row during planting at rates of 13 or 31 kg N/ha. Soil descriptions are given in Table 1.

Table 1. Soil characteristics

Depth		Wentworth sicl*	Nicollet cl*
cm			
0–15	pH	6.1	6.1
	CEC meq/100 g	30.1	27.2
	C %	2.70	2.55
	Clay %	30.0	26.6
	Silt %	63.4	33.4
	H_2O %		
	1/3 bar	44.3	27.0
	15 bar	26.8	13.5
> 30			
	1/3 bar	32	24
	15 bar	21	12

* Wentworth sicl = Udic Haplustoll, fine-silty, mixed, mesic at Madison, South Dakota. Nicollet cl = Aquic Hapludoll, fine, mixed, mesic at Lamberton, Minnesota (USDA).

Plant height measurements were taken periodically and representative plants were randomly taken for analysis. Plant N was determined using macro Kjeldahl[3]. Plant N uptake was plotted against time and correlated with a tanh (time) function iteratively until a maximum correlation coefficient was obtained. The function

$$N_u = \left(\frac{N_h - N_i}{2}\right) 1 + \left\{1 + \frac{e^{k(t-t_0)} - e^{-k(t-t_0)}}{e^{k(t-t_0)} + e^{-k(t-t_0)}}\right\}$$

differs from that given by Richards[11] in that N_h, the total N in the plant at harvest, is reduced by N_i, the N contained in the seed; N_u = N accumulated at any time, t; t_0 = time required to reach the maximum uptake rate and k = a time coefficient perhaps sensitive to the environment (temperature, moisture, light, nutrient supply, *etc.*). The derivative of the N uptake function, $sech^2$(time), was used to obtain estimates of maximum N uptake rates; these were regressed against applied fertilizer N to obtain uptake rates without fertilizer.

Soil cores were taken at planting and after harvest to a depth of 150 cm, sectioned at 15 cm

intervals and subsamples extracted with 1 M KCl. NH_4^+-N and NO_3^--N plus NO_2^--N were determined colorimetrically[15]. Soil moisture was determined to a depth of 150 cm biweekly with a neutron moisture meter.

Results and discussion

Tillage effects on plant growth and nutrient accumulation were more apparent on the Nicollet cl than on the Wentworth sicl. Without tillage, plant growth was generally retarded on the cl. Plant height was closely correlated with dry weight (exponentially) and % total Kjeldahl N (negative, linear) over the first 60 days after emergence, but, height relationships disappeared as the plants neared the reproductive stage. Plant N accumulation was inhibited with reduced tillage on the cl but not on the sicl; so, although many characteristics of these soils are similar, the plant-tillage responses show marked contrast.

Plotting N accumulation as a function of time, a typical S-shaped curve is obtained which can be closely fitted with a tanh (time) function[11]. From the derivative of this function, the sech^2(time), we obtain estimates of the rate of N accumulation at any time, t. Examples of both types of plots for a single tillage practice are given in Figs. 1 and 2.

Tillage had minor and apparently only indirect effects on the time required to reach maximum N accumulation rates, t_0 (Table 2). Mechanical difficulties on the sicl site in 1981 resulted in less dense stands

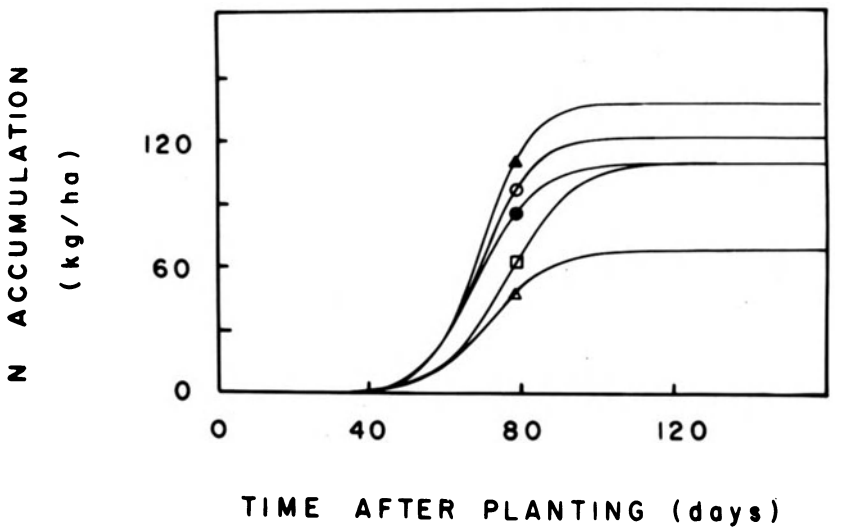

Fig. 1. Effect of fertilizer N application on N accumulation by maize from untilled Nicollet cl. The symbols △, □, ●, ○, and △ are not data points; they identify treatments of the fall broadcast applications of 0, 45, 90, 134 and 180 kg urea-N/ha, respectively.

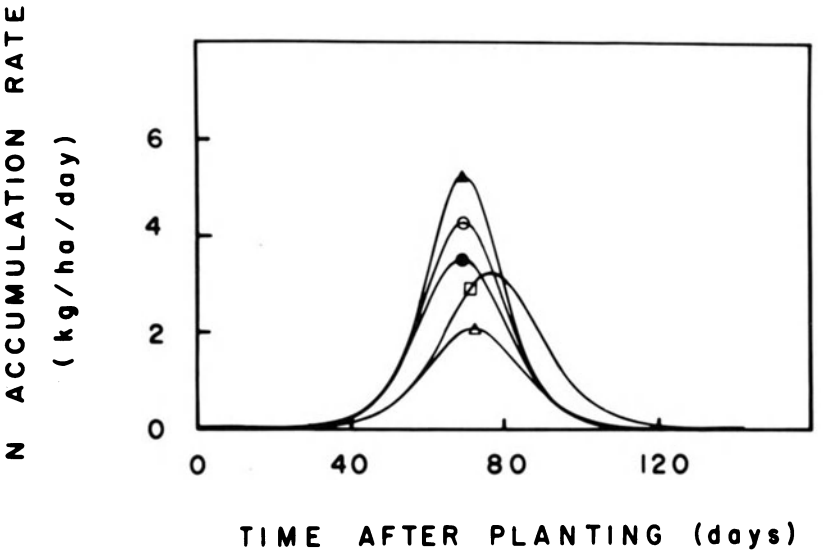

Fig. 2. Effect of fertilizer N applications on rate of N accumulation by maize from untilled Nicollet cl. Symbols are the same as in Fig. 1.

Table 2. Time, in days after planting, required to reach maximum nitrogen accumulation rate

Tillage	Nicollet cl*	Wentworth sicl*
	$\bar{x} \pm \bar{s_d}$	$\bar{x} \pm \bar{s_d}$
NT 1980	71.7 ± 3.1	72.7 ± 3.0
1981	65.4 ± 2.4	82.0 ± 4.5**
FC 1980	72.6 ± 3.0	70.3 ± 2.6
1981	69.1 ± 5.0	76.1 ± 5.5**
FP 1980	70.6 ± 3.9	75.0 ± 4.5
1981	75.2 ± 2.8***	75.6 ± 3.1

* Each mean consists of 15 determinations as Nicollet cl and 16 determinations on Wentworth sicl.

** Planting difficulties caused a 33 and 15% reduction in stand establishment on NT and FC treatments, respectively, relative to FP treatments.

*** Early spring drought caused a 10- to 14-day delay in mean plant emergence for the FP treatment.

and less inter-plant competition appears to have permitted a longer period of N accumulation. Early spring drought delayed plant emergence from FP plots on the cl in 1981 causing relative delays in maturity throughout the season. Both growing seasons were characterized by greater drought stress than that most frequently observed.

N accumulation rates at both sites were highly correlated with applied N for all tillages in 1980 but only for FC and FP treatments on the sicl in 1981 (Table 3). Drought in 1981 affected plant growth at both sites but the effects appear to have been more severe on the cl which has a lower range of available soil water contents. Without added

N, tillage increased maximum N accumulation rates by 15 to 80% on the cl but FP tillage decreased it by $\geqslant 25\%$ on the sicl. The results show that tillage of the sicl either had no effect or hindered the N accumulation process. However, because different hybrids were used at each site, interaction effects cannot be ruled out.

Maximum N accumulation rates tended to converge at about 110 ± 15 mg N/plant/day for all tillages as fertilizer rates increased to about 200 kg N/ha. Some absolute maximum N uptake must exist, so apparent linear relationships between fertilizer rate and plant N uptake are coincidental and restricted to low rates of applied N. Assuming (1) NO_3 is the main N uptake form, (2) all soil NO_3 is in solution, (3) a NO_3-N concentration of 6.5 mg/l, and (4) no diurnal effects, a transpiration rate of nearly 171 of water/plant/day is required to satisfy plant N accumulation rates if mass flow is the only means of transport, that is, about 90 mm water/m^2/day with a plant population of 50,000/ha. Liao's[7] observation of accelerated NO_3 uptake reduces the required water flux to about 15 mm/day – a high but more reasonable value.

Some explanation for differences in plant response to tillage is given in the soil mineral N contents. The sicl often contained a greater mineral N concentration than the cl. Both pre- and post-season soil NH_4-N concentrations were unaffected by tillage. NO_3-N concentrations in the sicl, however, were often decreased by 10 to 15 μh/g in the 0 to 30 cm zone of fertilized NT and FC treatments relative to FP treatments, whereas concentrations below this zone were unaffected by tillage (Table 4). Apparently, effects of tillage changes in the sicl on the soil mineral N concentrations are too small to affect plant N accumulations.

Table 3. Tillage effects on the maximum daily N accumulation rate by maize (mg/plant/day)

	Nicollet cl			Wentworth sicl		
Tillage	Without fertilizer N	Fertilizer N coefficient*	r	Without fertilizer N	Fertilizer N coefficient*	r
NT 1980	36.4	0.364	0.889	67.6	0.341	0.727
1981	78.5	0.070	n.s.†	88.0	0.179	n.s.
FC 1980	43.7	0.317	0.856	79.1	0.347	0.509
1981	90.6	0.109	n.s.	43.8	0.394	0.767
FP 1980	64.5	0.194	0.738	53.9	0.399	0.750
1981	89.3	0.097	n.s.	40.6	0.277	0.792

* Example: On Nicollet cl NT treatments in 1980, the maximum daily N accumulation rate in mg/plant/day was $N_u = 36.4 + 0.364$ (kg N applied/ha).

† n.s. = not significant ($p < 0.95$) students' t test results of comparisons between the regressed rate and a zero rate (Steel and Torrie[12]), i.e., that the slope of the regression equation is zero or unaffected by applied fertilizer N.

Table 4. 1980 post harvest profile nitrate-N concentrations (μg/g) in a Wentworth sicl

	Depth (cm)*	
	0–15	15–30
122 kg N/ha: No till	5.63b	4.88β
Fall Chisel	5.28b	3.75β
Fall plow	17.05a	18.85α
31 kg N/ha: No till	5.25α	3.98b
Fall chisel	5.75α	3.53b
Fall plow	6.33α	5.00a

* Values followed by the same letter within a depth and a fertility treatment are not significantly different ($p \geq 0.05$).

NH_4-N concentrations in the cl profiles show a few complex tillage v fertilizer interactions of which all but one occurred at depths below 60 cm. In all cases, concentration differences were relatively small, about 2 to 3 μg/g. Autumn NH_4-N concentrations were nearly double those of comparable spring profiles in the 0- to 30-cm zone and may reflect the plant-soil-climate interactions.

NO_3-N concentrations in the cl profiles showed complex tillage and fertilizer interaction effects below 100 cm in the spring. A distinct tendency exists for greater NO_3-N concentrations in surface fertilized FP treatments before planting and smaller concentrations in the 30- to 60-cm zone of unfertilized FP treatments after harvest. Also, NO_3-N concentrations are significantly greater in the post harvest FP treatments below 105 cm by about 1 to 3 μg/g relative to FC or NT treatments.

Even during periods of relative drought stress, untilled profiles tend to maintain about 2 to 5% greater volumetric water contents in the 0 to 120 cm zone than tilled profiles. Tillage v fertilizer interaction effects were observed in the cl, but they were not significant in the sicl. In the cl, additions of 120 kg fertilizer N/ha increased water use in untilled profiles to the point that volumetric water contents were nearly equal in all tillages when plants began florescence.

Observations of hybrid v tillage interactions are rather rare. Ciha[4] has noted tillage v hybrid interaction effects on spring wheat yields and recently a tillage v hybrid interaction effect was observed in maize on the Wentworth sicl[10]. With less aeration and changes in relative concentrations of oxidation-reduction sensitive plant nutrient forms, a potential exists for further development of hybrids which will perform better on some soils.

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Section 2

Microbial and animal effects on soil fertility

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The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil

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Key words Microorganisms Mycorrhizas Nitrogen fixation Phosphate uptake Rhizosphere

Summary No root systems in nature are without a microbial population. These may be free-living or symbiotic.

The incidence and nutrition of the freeliving microorganisms is discussed. Shortage of substrate makes it unlikely that the N-fixers in the population can fix useful amounts of N. There is a possibility that P supply is improved, but an analysis of possible processes shows them to be rather unlikely, and evidence for them to be poor. Manganese and iron uptake can be altered by microbial activity. Growth of plants can be affected by non-nutritional bacterial effects.

The ecology of Rhizobium in the soil is briefly discussed, and the varying needs of different identified strains is stressed.

Mycorrhizal infection of plants leads to large growth increases in appropriate conditions. This is almost always linked to increased P uptake, but zinc and copper nutrition can also be improved. The processes involved are briefly discussed. Rapid and extensive infection is important; it is very sensitive to temperature. New modelling methods are now becoming available to measure the behaviour of the fungal infections. The microorganisms require C compounds from the plant, and new measurements of this cost are discussed. The possibility of practical use of mycorrhizal fungi seem to be improving.

Introduction

No natural vegetation systems, and very few artificial ones, are sterile. Real-world plant nutrition occurs always in a complex system of plant, substrate and large numbers of different microorganisms. The first aim of this paper is to summarise the ways in which the microorganisms may affect plant nutrition. However, not all possible processes are important, and this subject has been particularly dogged by non-quantitative speculation on what may happen. The second aim is therefore to say what is likely to be important in practice. Although there is an abundant literature on the subject it is still not easy to say exactly why the 'rhizosphere' is important.

The most obvious distinction to make is between free-living organisms, which may complete their life cycle away from the plant, and those which, at least at some stage, are totally dependent upon the host plant. The first group may well increase greatly in presence of a plant root, but they normally live as saprophytic soil organisms. There have been many papers listing such microorganisms but little of this taxonomic work about the specific properties of different organisms in

terms of microbial ecology and competition, and the consequences of these for the plant.

Understanding of the symbiotic systems of the rhizosphere has expanded more rapidly. These include the N-fixing symbioses, such as those of Rhizobium and some Actinomycetes, and the mycorrhizas. Their relationships with the host are far more intimate and may be very species-specific.

Free-living microorganisms

Nutrient supplies to microorganisms

The distinction between symbiotic and non-symbiotic systems lies mainly in the ability of the former to obtain C nutrition from their hosts in a very specific way, details of which are discussed later. Non-symbiotic organisms obtain their nutrition from the additional C substrate in the rhizosphere, and are thus basically normal heterotrophs, but living in an environment in which the nutrient supply has beem temporarily much improved. Such microorganisms therefore tend to be able to adapt rapidly to improvements of nutrient supply, or to be 'r-type' organisms[48]. They develop on 'exudates' and other C supplies in the rhizosphere.

Rovira *et al.*[57] discussed and defined the nutrients present in the rhizosphere, including sloughed-off debris, contents of dying cells, as well as the soluble, low-molecular weight compounds more normally regarded as 'exudates' from a healthy root. The latter comprise very many compounds, mainly sugars, organic acids, and amino acids. It is virtually certain that the amount and composition of exudates varies with growth conditions[48], so it is extremely difficult to generalise about the substrate available to rhizosphere bacteria. If we assume that N, P and other elements can be obtained as inorganic ions from the main body of the soil, the primary question concerns the total amount of combined C supplied[70]. Two factors make this difficult to determine. Firstly, root growth and C loss is greatly affected by the medium in which the root is growing[42] and realistic estimates are only obtained from roots in soil media. Secondly, C loss from sterile roots is always less than from normal non-sterile ones, so axenic culture gives misleading results[5]. This is supported by the report of Asanuma *et al.*[4] that non-axenic roots had far more ruptured cells on their surfaces. Thirdly, it is very difficult to separate root respiration from the respiratory CO_2 produced by the microorganisms themselves. For these reasons the published values have varied widely, and the whole subject is in some confusion. Only experiments which employ ^{14}C labelling and

soil media seem likely to produce dependable results for soil grown plants, and even ^{14}C does not distinguish between bacteria-produced and root-produced CO_2.

Newman[48] concluded that soluble exudates would normally be in the range of 1–10% of total photosynthesis by the host plant. Measurements in axenic solution culture usually give 1–2% at the most, and it may be that this figure is only modestly increased in non-sterile soil. However, larger amounts of solid root debris must be produced because work with ^{14}C (Barber and Martin[6]) suggests that C equal to as much as 25% of net photosynthesis may be lost from the root surface.

The total energy supply must affect the rhizosphere biomass greatly. Newman and Watson[49] have made by far the most detailed attempt to predict behaviour in the rhizosphere on this basis, using a computer simulation model, and parameters drawn from measurements on bacteria in culture. However Brookes *et al.*[12] have shown that the soil micro-organisms are on average dividing slowly, though they have a remarkably high adenylate energy charge, so their state is different to that of organisms grown in chemostats. When bacterial numbers expand rapidly in the rhizosphere, the conditions may be closer to those in a chemostat than to those in the bulk soil, but it does raise doubts about using data from organisms growing in non-soil media to predict the conditions in the rhizosphere.

Effects on higher plants

Barber[7] has reviewed the subject in detail. Four major effects are considered here as follows:

1. *N fixation and its C cost* Rhizosphere organisms certainly include N-fixers of the genera Azotobacter and Azospirillum[50]. There have been claims that important amounts of N can be fixed in the rhizosphere, or even in the root cortex, by such organisms. The principle is not in doubt, and the debate is simply about the probable importance of this process. In this context, the need for C by all N-fixers is important, because it sets the limits within which the process has to work. It is discussed here for both the free-living organisms and Rhizobium.

For Rhizobium, the cost has been given as about 6.5 g C g^{-1} N[54]. The essential process is the transfer of a pair of electrons to a nitrogenase substrate, either dinitrogen or acetylene, but in addition there is the production and maintenance cost of the nodule and its contents. The actual cost of the N reduction can now be measured from the changes in CO_2 production association with the reduction[77]; it is 2–5 moles CO_2 per mole C_2H_2 reduced. For N, this equals 3.5–9 g C g^{-1} N,

depending upon the symbiotic host and Rhizobium strain, in fair agreement with the value quoted above. This implies that, for 1–2% N in dry matter, and a cost of 6.5 $gC\,g^{-1}$ N, some 13–26% of the dry weight of the legume may be needed for fixation of N. However, good experimental evidence that the C cost of N fixation will decrease crop yield below what otherwise could be obtained is difficult to get. Barber and Lynch[5] first made a simple but devastating calculation, in which they showed that the amount of C released from the roots was unlikely to be sufficient for any useful fixation. If we take an estimate of C release as 20% of total photosynthate, and the efficiency of fixation as quoted above, it appears that some 1.5% N of total dry weight could be fixed, if the whole of the released C were used for this purpose, at the same efficiency as in legumes. The symbiotic endophyte Rhizobium at least has good access to the C produced by the host. Associative N-fixers, such as Azospirillum in the rhizosphere, are dependent upon the exudate from the root, and competition for this is intense because of the rapid increase of the general bacterial population[49]. Free-living N-fixing bacteria are also inefficient because of their large need to use C for respiratory protection of the anaerobic condition of nitrogenase. As the N-fixers will get only a fraction of the total C available it seems that the contribution from non-symbiotic fixation must be quite small under the best possible conditions.

Early estimates of non-symbiotic fixation were often inflated, being based on measurements of acetylene reduction in soil cores, multiplied up to a hectare basis. More recent work has rarely suggested fixation rates above about 30 $kg\,N\,ha^{-1}\,yr^{-1}$, which may be important for natural vegetation, but is of little import for intensive agriculture. Lethbridge and Davidson[40] found that four microorganisms on wheat roots reduced acetylene strongly, but no detectable amounts of fixed N were formed in the plants. However, when the root medium (sand) was enriched with organic materials artificially, some 15% of the N in the plants was provided by fixation.

The present position is thus that non-symbiotic fixation of N by soil organisms can undoubtedly occur, but there is no consistent and accepted evidence up to the present that it is of much value for agricultural crops. The strongest evidence for significant fixation is perhaps from work on the Broadbalk 'Wilderness' plots at Rothamsted, in which average accumulation rates of 39 $kg\,N\,ha^{-1}\,yr^{-1}$ were found in the absence of legumes, after allowing for rainfall input, but other estimates are a good deal smaller[14].

2. *Plant hormonal effects.* It is possible that the properties, morphology or growth of the plant could be altered by bacteria. There now

appears to be no doubt that bacteria on the root surfaces can improve growth rates, by up to 10% or so[13]. The mechanism does not generally appear to be directly due to nutrition, but rather compounds with plant hormone activity[41]. Such bacteria may also be effective by acting as antagonists to weak soil-borne plant pathogens[33]. If growth is simply enhanced, the larger plants will of course contain more nutrients, but this is an effect rather than a cause.

More interesting from our point of view are firstly, changes in root morphology, and secondly in their uptake properties. The most important and distinct effect on root morphology is on root hairs, which may become less in length and number in non-sterile media[9], but Asamuna *et al.*[4] found the opposite result. Unfortunately no work appears yet to have been done to show whether or not such effects are related to the presence of specific organisms. Mosse[43] has discussed these effects at greater length.

3. *Increase or decrease in P supply* The possible mechanisms which have been suggested at one time or another, are:

A. Effects in growth medium.
   1. Alteration of solute concentrations ('solubilization') by pH change, by chelate production, or by organic anion production
   2. Direct competition in uptake of P

B. Direct effects on plant.
   1. Alteration of root uptake properties by: a) effects on uptake mechanism and b) change in relative growth rate, or internal composition
   2. Alteration of root morphology

A1. Suggestions on 'solubilization' have a long history (see Barber[7], Tinker[71]). Briefly, it is clear that appropriate bacteria can certainly cause poorly soluble phosphates to dissolve in pure culture. However, it is improbable that such effects could be obtained in soil which is buffered, in which the supply of cations to be chelated is very large, and in which phosphate-competing anions are rapidly sorbed. No papers suggesting such processes appear to have attempted to quantify how much bacterial activity or production of material would be needed. Nye and Tinker[51] (p. 172) discussed these effects in more quantitative terms, in relation to the possible amounts of microbial compounds being produced. The over-riding factor is that these effects could mostly be produced directly by the plant itself, and in some cases such as pH change, to a much larger extent than any conceivable microbiological process (see Tinker[71], Hedley *et al.*[31]). The burden of proof appears to rest firmly on those who propose that such mechanisms *are important.*

A2. Direct competition can only be important if supply is limited, as may happen in a solution culture of limited size, or in a nutrient deficient soil. The data of Barber and Lynch[5] suggest 210 μg dry weight bacteria per mg root-dry weight as critical. If bacteria contain 1% P on average, this is 0.21% P to root dry matter. As the tops will normally be 2–10 times as large as roots, this does not appear a very large loss for the plant. In the soil the bacterial biomass should be broken down more rapidly, so it seems unlikely that competition will make much difference to the growth of higher plants.

B1. Root-uptake properties. There is little doubt that bacteria can directly alter root uptake properties, as shown in short-term uptake experiments by Barber *et al.*[6]; translocation to the shoot was also altered. However, it seems unlikely that it is solely the properties of the root uptake systems which control uptake in the longer turn, but rather the plant demand which adjusts these properties. Any change in root uptake properties over a considerable period really implied a change in the relative growth rate of the plant, or its composition, according to the equation[51]:

$$I = W/L \cdot P \cdot Rw$$

where I is inflow, W dry weight, L root length, P the fractional nutrient content and Rw the relative growth rate. Thus any effect on the growth rate of the plant, or upon its elemental composition can be seen as a change in its nutrient demand, and hence in its root uptake processes.

B2. If the soil-root system is such that changes in root morphology can alter the uptake rates then the effects discussed above due to rhizosphere bacteria will change the nutrient uptake rate also[51].

4. *Effects of rhizosphere bacteria on uptake of manganese and iron*
These are well substantiated[7]. The presence of a heat-labile bacterially produced substance increases root uptake of manganese. In soil, additional effects have been shown, in particular the enhanced oxidation of divalent manganese to the tetravalent, much less available form, probably by an enzyme produced by soil bacteria[22]. Analogous effects, but which are much less well agreed, have been reported for molybdenum and iron. Recently bacterial compounds with a very strong affinity for iron have been described and named siderophores[23], but their significance in plant nutrition is as yet uncertain.

Rhizobium

These are gram-negative bacteria, which are able to live as heterotrophs in the soil[74]. Their distinctive characteristic is the ability to

infect root cortices, by the growth of an infection thread down the root hairs of leguminous species. The infection thread is formed from plant material, and the ability of the bacteria to organise and control plant growth is the essence of this symbiosis. This bacterial infection then invades from the root hair, across cell walls. Meristematic activity begins in the cortex, a nodule forms, and rhizobia are released from the infection threads, into the host tissue, as 'bacteroids'. The morphology of the host cells is changed, and some become polyploid. The 'bacteroids' contain nitrogenase, and fix N into ammonia, which is then transferred to the host plant.

The amount of work on Rhizobium is so vast that it cannot be summarised even briefly here. I intend to concentrate upon some of the factors controlling it in the soil and the rhizosphere, and upon the cost of the symbiosis to the plant.

Ecology in the soil

Rhizobium can live freely in the soil, but numbers are always relatively low compared with those of other rhizosphere inhabitants. Their ability to infect legumes is improved by their mobility in soil with low water tensions[34], movements of up to 2 cm day^{-1} have been observed. However, non-motile mutants can also infect legumes in the soil, so motility is not a precondition of infectivity. There is clear evidence of stimulation of Rhizobia near to roots, and chemical tropism is likely, due to root exudates[38]. The extent of the stimulation may be correlated with the ability of that strain to form nodules with that particular host[53]. In the rhizosphere of such successful hosts, numbers of Rhizobia can rise to 10^7 g^{-1} soil. In addition to this stimulation, there are extensive parasitic and competitive interactions with other organisms in the rhizosphere[53]. In particular, a very important aspect of competition is whether effective strains of Rhizobia (in terms of N fixation) can compete with ineffective ones during infection.

An important factor in the efficiency of the Rhizobium symbiosis is the P supply. Because N fixation is so dependent upon the level of P supply there are strong interactions between the infections of legumes with Rhizobium and with VA mycorrhizas[46]. It is as yet uncertain whether this interaction is solely via P supply, or whether other processes are involved. It is very interesting in this context that different strains of Rhizobium have clearly defined differences in P requirement[18].

The differences in nutritional and soil requirements of Rhizobium strains are highly important to their ecology, and their use. Aluminium tolerance in particular is widely variable between strains, but stable

within a given strain[45]. Very recently, Witty *et al.*[77] have measured the C demand by different Rhizobium strains, and showed that the C use per unit of N fixed can vary by a factor of 2. In view of the discussion on C demand earlier, this may be a point of major importance in selecting efficient endophytes.

These clear differences between well-defined strains of Rhizobium are particularly important now, because of the recently developed possibility of inserting genetic information into the bacteria which offers the possibility of bringing together favourable properties within a relatively short period[32]. However, merely breeding 'elite' strains of Rhizobium is unlikely to solve all problems. These still have to operate within the normal laws of physics and chemistry and the biology of the host plant. More attention to the morphology and physiology of the latter is needed to complement present work on Rhizobium alone. For example, careful attention to the agronomy allowed Blanca clover to fix 440 kg N ha^{-1} at Rothamsted[35], a figure far larger than the average value for temperate zone agriculture[39].

Mycorrhizas

General biology

Mycorrhizas are usually classified, on the basis of appearance, as ectomycorrhizas or endomycorrhizas, with a few cases of intermediate form. The endomycorrhizas are the most important in terms of the species they can infect, and include the ericaceous and the vesicular-arbuscular mycorrhizas. Ectomycorrhizas infect almost solely forest trees, the ericaceous mycorrhizas have a fairly limited host range, but the vesicular-arbuscular (VA) mycorrhizas are almost ubiquitous, with an enormous number of host species, including many major crop plants. Ectomycorrhizas form a distinct sheath around the host with a network between the cortical cells, but most also have a mycelial network in the soil. Ericaceous and vesicular-arbuscular types inhabit the root cortex, and produce specialised bodies (coils, arbusules, vesicles) within or between the cells, and these always have an extensive network of hyphae in the external medium[26].

Ecology

The effect of environmental factors is known fairly well in general terms, but much more precision would be desirable[44]. Information on differential sensitivity to pH and soil nutrient levels is still rudimentary. VA mycorrhizas in particular are temperature-sensitive, both in spread and function. Whether infection is measured in terms of infected root

length[27] or entry points[61], it appears that spread through a root system accelerates rapidly above 10–20°C. Direct measurements of the uptake and translocation of P by hyphae[19] showed that activity decreased sharply below 16°C, and almost ceased at 8°C. There is thus a good deal of evidence about the sensitivity of VA mycorrhizas to low temperatures, which explains their slow infection in spring, and their predominance in warmer climates. Less attention has been given to the top end of the range, but development and function appears to decrease above about 30°C. Much work on temperature effects in ecto-mycorrhizal fungi in pure culture has shown that they also grow best at temperatures above those normally encountered in the field in the temperate zone[44]. However, little of this work has dealt with infection as a dynamic process. This is important, because in terms of the nutrition of the plant, it is the relative rates of growth of the fungus and the host which determine effective levels of infection.

It is known that waterlogged conditions may slow or prevent mycorrhiza formation, possibly because of the difficulty of meeting the oxygen demand of a fungal network in soil or cortex, when the soil is waterlogged and the root depends on internal diffusion of oxygen. Infection appears most rapid at intermediate water contents[56].

Phosphorus uptake

The most clearly agreed function is to improve the supply of P to the host (see Tinker[71]; Hayman[29]). This can result in greater growth, higher % P, or both. In effect, the P response curve of the plant is displaced, so that it acts as though the P status of the soil were greater[65].

The mechanism is now fairly well agreed and need not be discussed in detail here (see Tinker[68]): briefly, the hyphae are able to absorb P from the soil well away from the host. Arguments based on diffusion theory show that the uninfected root could not absorb as fast as a mycorrhizal one. At present there seems little doubt that hyphal uptake is the main mechanism for increasing P uptake, with subsequent conversion to polyphosphate, translocation to the arbuscule and transfer to the host.

There are however other theories. Cress *et al.*[20] suggested mycorrhizal roots have different uptake properties, and in particular a lower Km value than non-mycorrhizal ones. There are weaknesses in this argument (see Tinker and Gildon[67]), but it would be surprising if Km and Vmas were identical with and without mycorrhizal infection. Mycorrhizal roots may thus act differently from non-infected ones even without wide distribution of external hyphae, and it has been suggested that this allows mycorrhizal roots to absorb from concentrations so low that

non-mycorrhizal roots absorb nothing. It is possible that mycorrhizal roots exude more chelating acids, which may enhance uptake[68], but no evidence for this is yet forthcoming. In this context it is of interest that mycorrhizal roots have a different cation-anion balance to that of non-infected roots, and this may be relevant to altering the pH of the rhizosphere[17, 60] has proposed a model whereby the internal pH regulation is affected by mycorrhizal infection.

Very large increases in P inflow have been measured, up to 4 or 5 fold, and growth responses of up to 100 fold have been reported. The response is usually related to the response to application of P, though mycorrhizal infection will not, of course, always cure P deficiency; this depends upon the degree of the deficiency and other conditions. There has been particular interest in the possibility that infection can improve uptake from phosphates of low solubility. It seems clear that this can happen, but that infection is of little use if the source is so insoluble that the uninfected plant cannot use it at all[70].

Finally, there are several reports that infection with mycorrhizas will change the hormonal condition of the plant, which may affect its water or nutrient status (*e.g.* Allen *et al.*[3]).

Trace metals

It has long been known that mycorrhizal infection could alter the zinc uptake of plants (see Tinker[69]). More recently this has been proved quite explicitly for copper also[25, 66]. The mechanism is not known in detail, but VA hyphae have been shown to translocate zinc, so we assume that the mechanism is similar to that for P.

In addition to supplying metals at low concentration, there are now interesting results on the effects of mycorrhizas in presence of high concentrations of heavy metals. These are toxic to the fungi, and can greatly reduce infection[67]. From this one would expect some interaction with the growth of the host, which needs further work.

Bradley *et al.*[11] have shown that infection can markedly reduce the damaging effect of heavy metals on Calluna (heather). Whatever the mechanism, the effect is of considerable ecological and physiological importance. However, very recently Killham and Firestone[36] have reported that VA mycorrhizal plants contain much greater concentrations of heavy metals than nonmycorrhizal but only where they are exposed to 'acid rain'. Tinker and Gildon[67] found only small effects of mycorrhizal infection on heavy metal content, so the situation is currently confused.

Infection development and modelling

The mycorrhizal development in a root system has normally been measured by percent infection of the total length. This simple measure has been used to compare different endophyte species or isolates, to determine the effect of environment variables such as temperature, and as a measure of the 'success' of inoculation. It has been very successful in this role, and the rapid lengthening of infected root tissue may be of basic importance in the functioning of the symbiosis[1, 58].

It has been less clear what controls development of infection with time. The spread of the fungus through a root system which is itself growing is a highly dynamic process and single-harvest experiments give partial or even misleading results. For this reason there has been much recent interest in developing mechanistic models of VA infection development[16, 62]. Developments of this type will allow the 'efficiency' of symbiotic systems to be analysed in much greater detail than at present. For example, a 'good' endophyte could germinate rapidly, infect rapidly, spread rapidly through a root system, produce much external mycelium, be particularly effective at absorbing or transferring P, or be very economical in its use of host C. A partial analysis of this type is in the work of Sanders *et al.*[58] and Wilson and Trinick[76]. Sanders (private communication) has fitted such models to the development of infection with various host-endophyte combinations, so that their properties can be described by one or two parameters, and this appears to be the most profitable way to define the value to the host.

Carbon cost

Just as with the non-symbiotic organisms and Rhizobium, the question of C cost must be considered. Pang and Paul[52], Kucey and Paul[37] and Snellgrove *et al.*[64] agree on a C cost of the order of 4–10% of total photosynthate. However, Silsbury *et al.*[59] were unable to detect any difference in the C economy of clover after infection. It seems possible that the C demand is a function of the activity of the endophyte, and not just of its presence. A variety of reports of reduced yields after VA infection have been discussed by Tinker[69], and are linked to the possibility of a drain on host photosynthate[15]. In fact both Pang and Paul[52] and Snellgrove *et al.*[64] found that infected plants appeared to compensate for the drain. The analogies with the C demand of Rhizobium are particularly interesting.

Practical use of mycorrhizas

There are several well-substantiated cases of marked growth response of forest trees planted outside their normal habitat when inoculated

with their normal symbiotic fungi. Such cases for the VA and ericaceous mycorrhizas are far fewer, though much effort is currently being directed to this subject. Methods of inoculation have been tested extensively and good responses on sterilized soils have been found[55, 78]. But among the native soil fungi there are usually adequate symbionts and there is a lack of host specificity, many different fungal strains or species can give benefits similar to inoculants. Thus practical application of knowledge of the VA or ericaceous mycorrhizas has not developed as rapidly as could have been expected[72]. However, prospects are now appearing better for practical use of inoculation, in areas which have naturally low inoculum, in soils which have been radically altered e.g. by drainage or liming, and in soils or systems in which sterilization is extensively used. Abbot and Robson[1] and Hayman[27] have discussed in detail the methods to be used in testing and using these fungi, and methods of producing commercial inoculum are now being developed. The ability of mycorrhizal infection to spread from the original inoculum could then be important[75].

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Earthworms and soil fertility

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Key words Biological effects Earthworms Fertilizers Lime Nutrients Physical effects Plant growth Roots Soil fertility

Summary Earthworms redistribute organic materials within the soil, increase soil penetrability and, under certain conditions, influence ion transport in soils. Root distribution may be modified and microbial activity increased by their burrowing and feeding activities. Earthworms influence the supply of nutrients in several ways. Not only is earthworm tissue and cast material enriched in certain nutrients, relative to the soil matrix, but ingestion of organic material increases the rate of cycling. Certain farm-management practices, such as cultuvation and the use of acidic fertilizers, reduce the ability of earthworm to improve plant growth. Where other inorganic fertilizers increase the growth of plants, an increase in earthworm numbers can be expected because of the increased food supply. Lime, in particular, and possibly drainage also increase earthworm activity. Further research is required on the physical and biological effects of earthworms on nutrient supply, so that suitable management practices can be developed to optimise the beneficial effects of earthworms on soil fertility.

Introduction

General statements about the importance of earthworms for soil fertility have been made since Darwin[4] but quantitative work on the mechanisms by which earthworms bring about changes in the soil is relatively recent. Also, several soil-related farm-management practices can affect the distribution of earthworms, which emphasises the interactive nature of the relationship between earthworms and soil fertility.

Most of the earthworms referred to in the literature are Lumbricidae, a group with a predominantly Palaearctic distribution. Within the Lumbricidae there is a great variation in the ecological requirements and effects of different species, some of which live and feed at the soil surface, while others burrow deeply into the lower soil horizons. There are many species belonging to the Megascolecidae in the tropics and subtropics, but the complexity of this group as well as its predominance in these latitudes means that little is known of the ecology of the many species or of their effects on the soils[10].

"Soil fertility" is a frequently-used but often poorly-defined term. Brady[3] gives a useful working definition which considers "fertility" to be *the inherent capacity of soil to supply nutrients to plants in adequate amounts and in suitable proportions.* Use of a nutrient basis for this definition does not imply that soil fertility has an exclusively

chemical basis because physical and biological factors can have a major effect on soil nutrient status. Earthworms can have important influences on physical and biological effects that affect the nutrient supply to plants and it is with these interactions, illustrated in Fig. 1, that this paper is concerned.

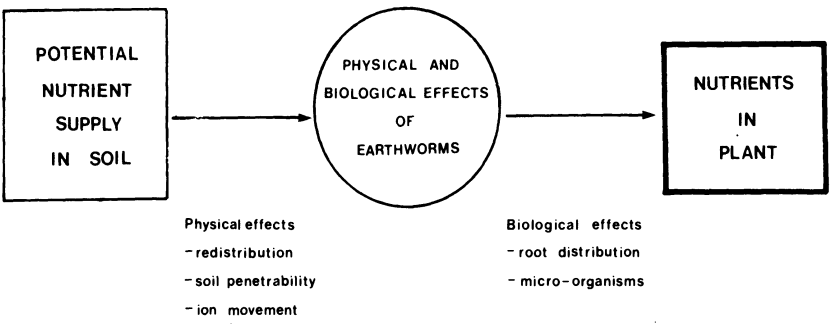

Fig. 1. Physical and biological effects on soil nutrient supply which are influenced by earthworms.

Effects on plant growth

Earthworms burrow through soil and feed on organic material, and so change the environment of all other soil-inhabiting organisms, including plant roots.

There have been numerous experiments in which plants have been grown in pots, with and without earthworms, where an increase in plant growth in response to earthworms has occurred. Experimental work suggests several different mechanisms by which earthworms can increase plant growth. Firstly, by increased incorporation of organic material[18], which may accelerate mineralisation; secondly, by a possible direct effect of earthworm metabolic products on plant growth[9]; and thirdly by improving aeration, water relations, and penetrability in poorly-structured soils (*e.g.*, McColl *et al.*[13]). None of these mechanisms has been conclusively isolated experimentally and it is likely that all operate simultaneously, the mechanism of major importance at any one time being determined by such factors as the initial chemical and physical nature of the soil, the crop being grown, and the species of earthworm present.

Physical effects of earthworms on nutrient supply

Redistribution

Some earthworm species selectively feed on plant residues at the soil surface (*e.g.*, *Lumbricus terrestris* L.) whereas others (*e.g.*, *Octolasion*

cyaneum (Sav.)) rely on decomposed organic material within the soil profile for their food source. Plant residues and dung are fragmented and mixed with soil during passage through the earthworm gut, and incorporation into the soil profile. This is an important step in the cycling of organic matter[21], redistributing nutrients in the soil and reducing their loss in surface runoff. Sharpley *et al.*[24] found that the amounts of dissolved inorganic P and NH_4-N and NO_3-N transported in surface runoff were between 4 and 8 times greater when earthworms were eliminated. Transfer of nutrients from the soil surface to zones where moisture is likely to be less limiting to root activity increases the effective use of nutrients. This is particularly important with materials of low-water-solubility, such as lime and phosphate rock.

Earthworm species vary in their ability to mix lime into soils[26]. For example *Allolobophora longa* Ude mixed lime to a much greater depth than *A. caliginosa* (Savigny) and *L. rubellus* Hoff. Also, the incorporation and intimate mixing of phosphate rock particles with soil by *A. caliginosa* and *L. rubellus* increased the agronomic effectiveness of a surface-applied, pelletised phosphate rock by 15 to 30% in a glasshouse experiment with ryegrass[14].

Soil penetrability

Compaction can impede root development in some soils, although this does not necessarily reduce crop yield[19]. However, if the availability of water and nutrients is limiting plant growth, the burrowing activities of certain earthworm species may be beneficial in allowing a greater effective soil volume to be exploited by roots. Photographic evidence of roots following earthworm channels in compacted soil indicates that earthworms are better able to penetrate soils of high "strength" than are plant roots. In the absence of earthworms, roots may be restricted to planes of weakness in such soils[19].

Ion transport

Channelling by earthworms and the opening of burrows to the surface may increase the rate of infiltration. However, unless the channels are in contact with free water they will have little influence because they will be filled with air. So in a soil which is sufficiently permeable it is unlikely that earthworm channels will have any significant effect on water and ion movement under natural rainfall.

When water ponds on the soil surface, preferential flow of water down channels created by earthworms can be important, as shown by Scotter and Kanchanasut[20] using solutes and dyes. However, there may actually be less leaching when preferential flow occurs than in a soil

where water movement is more uniform, as ions present within the soil matrix between preferential flow channels are relatively isolated from leaching.

The availability of nutrients to plants is influenced by the distance over which ions diffuse in the soil. Phosphate ions, in particular, diffuse over very short distances (less than 1 mm) and thus only P within 1 mm of a root surface is potentially available for uptake. If earthworms can improve root distribution and increase the volume of soil exploited (as discussed below), then limitations to nutrient uptake caused by reduced physical availability will be lessoned. This physical-biological-chemical interaction could be the overriding effect of earthworms on nutrient supply in some soils.

Biological effects of earthworms on nutrient supply

Root distribution

That earthworms increase the volume of root material penetrating the soil has been demonstrated by Edwards and Lofty[6] in the laboratory and by Stockdill and Cossens[27], Ellis *et al.*[8], and Edwards and Lofty[7] in the field. Edwards and Lofty[6] showed that the zone of maximum root growth of barley coincided with the zone of major activity of the earthworm species. Surface-active species promoted root growth in the surface soil layer whereas deeper burrowing species promoted deeper rooting. In field studies, Edwards and Lofty[7] demonstrated that cereal roots followed channels in the soil whether made artificially or by the activity of earthworms. Stockdill and Cossens[27] showed that in a soil devoid of Lumbricidae, the rooting depth of pasture species was severely limited but after inoculation with *A. caliginosa* and *L. rubellus* roots were able to penetrate more deeply into the soil.

Microbial activity

Although the total numbers of microorganisms in earthworm gut contents and casts depend on the initial food source, the greater the organic matter content the larger the microbial population. in all cases there is an increase in microbial population and activity during passage through the gut. Parle[17] showed that numbers of bacteria and actinomycetes increased 1000 fold during passage through the gut and oxygen consumption remained higher in earthworm casts than in soil for 50 days, indicating an increased microbial activity. This enhanced microbial activity is probably responsible for the increased phosphatase[22] (Fig. 2) and urease[29] activity found in earthworm casts

(particularly of *L. rubellus* and *A. caliginosa*), compared to underlying soil. However, it is probable that the interaction of earthworms and the soil microflora is more complex than mere mixing of microorganisms with finely-ground organic material.

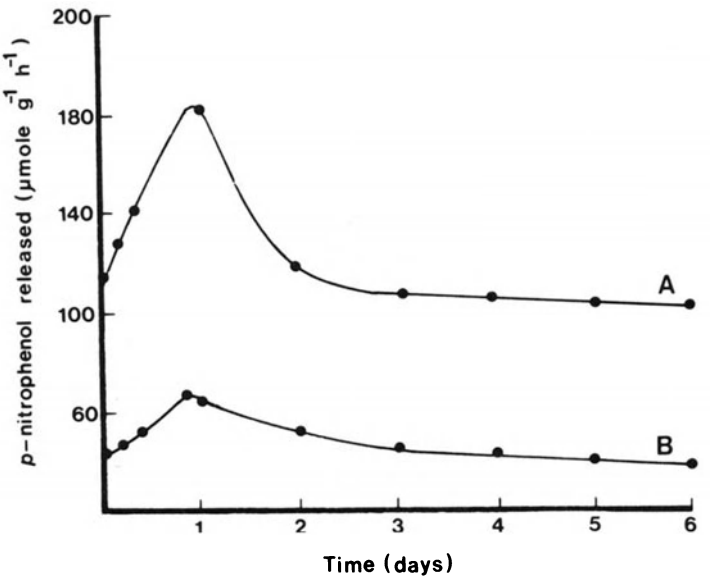

Fig. 2. Phosphatase activity, determined by *p*-nitrophenol released, as a function of time of incubation at 16°C of fresh casts (largely *L. rubellus* and *A. caliginosa*) (A) and underlying surface (0–50 mm) soil (B). Data from Sharpley and Syers[22].

Amount and rate of cycling of nutrients

Earthworms influence the supply of plant nutrients in the soil in several ways. The nutrients present in earthworm tissues are recycled as the earthworm population dies and young earthworms grow. Nutrients are cycled more rapidly through the earthworm's own metabolic processes and their feeding activities increase the release of nutrients.

Amounts of nutrients in earthworms

The chemical composition of earthworm tissue and the rate of release of N into the soil are shown in Table 1. The amount of N released annually into the soil, mainly as dead tissue protein and as excretory mucus, urea, uric acid, and ammonia, is estimated as being between 18 and 92 kg ha^{-1}, depending on the size and activity of the

Table 1. Chemical composition of earthworm tissue and rate of release of N to the soil (after Laverack[11])

Parameter	Biomass: standing crop in Autumn	Biomass: annual production	Metabolism: annual excretion as mucus, urea, uric acid, and ammonia
Liveweight	0.3–1.5 tonne ha^{-1}	0.3–2.5 tonne ha^{-1}	–
Dryweight	44–220 kg ha^{-1}	44–330 kg ha^{-1}	–
Protein	26–132 "	25–200 "	–
Fat	4.4–22 "	4.4–33 "	–
Carbohydrate	7–33 "	7–50 "	–
Ash	7–33 "	7–50 "	–
N	7–33 "	10–50 "	8–42 kg ha^{-1}

earthworm population. These figures suggest that while an earthworm population is increasing, as for example in a newly-established grassland, then the population will, on balance, be immobilising N. However, when a steady-state population is achieved the earthworm population should be making a significant contribution to the available N pool in the soil. Given that the protein content of earthworm dry tissue is approximately 60% (Laverack[11]; *i.e.* 10% N) and that the P and S content varies from 0.7 to 0.9% (McColl, H.P., personal communication), then a N:P:S ratio of about 12:1:1 is indicated. This suggests that the contribution of earthworm fauna to the annual cycle of P and S will be 2–9 kg ha^{-1} under New Zealand conditions.

Amounts and forms of nutrients in casts

The nutrient enrichment of casts relative to the soil matrix occurs because the organic materials ingested by earthworms contain a higher concentration of nutrients than soil. Because of their greater ease of collection, major emphasis has been directed towards the composition of surface casts.

An early study by Lunt and Jacobson[12] showed that casts from a ploughed soil in Connecticut, U.S.A., had approximately three times the concentration of exchangeable Mg, seven times of available P and eleven times of available K than the top 150 mm of soil. Recent studies[22,23] on the chemical nature of P in surface casts collected from a pasture soil in New Zealand have indicated that surface casts contain approximately four times more loosely-bound inorganic P and twice as much loosely-bound organic P as underlying soil. A greater isotopic exchangeability of inorganic P in casts was associated with the more extensive release of inorganic P from casts. The amount of loosely-bound inorganic P extracted from freshly-deposited casts increased as a function of time of incubation, while the release of organic P showed the converse trend, consistent with changes in phosphatase activity.

Similar differences in the amounts of extractable NH_4-N and NO_3-N between surface casts and underlying soil have also been obtained[29].

Rate and seasonality

By increasing the rate of cycling, earthworms increase the quantity of nutrients available at any one time. Consequently, direct effects of earthworms on nutrient uptake are likely to be more important in soils of low nutrient status[28]. This is illustrated by the data in Fig. 3 which show that the passage of ^{32}P-labelled ryegrass through earthworms increased the short-term plant availability of P by a factor of approximately three. When the casts were added to the surface of a sand or soil, recovery of ^{32}P from casts by ryegrass grown in sand having a very low availability P content was substantially greater than in soil with a higher available P content.

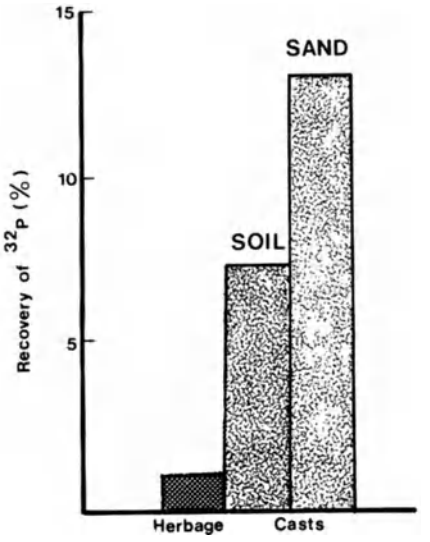

Fig. 3. Recovery by ryegrass grown in soil or sand of ^{32}P added in labelled herbage or in labelled casts (largely *L rubellus* and *A. caliginosa*) to the surface of the soil or the sand. Data from Mansell *et al.*[15].

Earthworms ingest dead plant material of varying but relatively wide C:N ratio and convert it to earthworm tissue of lower C:N ratio, the N of which is readily released on the death of the earthworm. Also, surface casts usually have a lower C:N ratio than underlying soil[29]. Both of these pathways of N mineralisation lead to an increase in the quantity of N available to plants at any one point in time and thus in the rate of cycling of N.

The rate of supply of nutrients to the soil from casting and excretion is determined by the feeding activity of the earthworm which is influenced by moisture and temperature, being greatest under moist and warm conditions which also favour plant growth. Earthworm mortality is affected by moisture and temperature but is greatest under dry or freezing conditions[2] when leaching losses are minimal. Under more favourable environmental conditions the nutrients contained in dead earthworm tissue, particularly N, may become available for plant growth.

Effect of farm-management practices on soil fertility and on earthworms

Farm-management practices which reduce the ability of earthworms to improve plant growth

In general, physical disturbance of the soil reduces earthworm numbers, although in some heavy soils or soils with a compacted layer, cultivation may be beneficial to earthworms. Ellis *et al.*[8] found that cereal crops which were direct-drilled had larger earthworm populations than ploughed crops. The degree of soil disturbance and the amount and distribution of organic matter remaining in the soil after harvest are factors which affect earthworm numbers. At Broadbalk, Rothamsted, Edwards[5] showed that the wheat cropped continuously for 136 years supported a larger population of earthworms than any of the rotations used at the same input of fertilizer. A wheat crop following potatoes and beans had a population about half that of the continuous wheat treatment but no earthworms were detected in a fallow following wheat. These data indicate the potential for recovery of the earthworm population. However, recovery to maximum population requires several years so that annual cultivation progressively reduces the population and prevents recovery.

Regular use of acidic fertilizers, such as ammonium sulphate, decrease earthworm populations[5] and maintain the soil at a pH which discourages reinvasion by worms. Several agricultural chemicals have a direct toxic effect on earthworms[5]. In general the nematicides and carbamates are highly toxic, as are some organochlorines (chlordane and endrin) and some organiphosphates (phorate and parathion). Herbicides tend to be less toxic.

Farm-management practices which increase the ability of earthworms to improve plant growth

There is abundant evidence from the Rothamsted experiments that the addition of organic fertilizer materials, such as dung, stimulates

certain earthworm species[5]. In situations where inorganic fertilizers increase the growth of plants then an increase in earthworm numbers can usually be expected, because greater plant growth provides more food for earthworms. This is particularly likely where non-acidic, nitrogenous fertilizers are used to stimulate vegetative growth, although it can occur with other nutrients. Barley[2] found that the addition of phosphatic fertilizer to pasture in Australia increased the weight of earthworms relative to the control. Significantly, superphosphate and ground phosphate rock gave the same increase in earthworm numbers[1].

In a well-fertilized, non-responsive situation, where fertilizers are added to balance nutrient losses, high earthworm numbers will improve soil physical and biological properties. The degree to which this affects plant growth will be influenced by the factors that are most limiting.

Earthworm species vary in their sensitivity to soil acidity. Addition of lime is known to stimulate certain species, particularly *A. caliginosa*. It has been claimed[16] that the numbers of *A. caliginosa* and *L. rubellus* in soils under pasture in New Zealand were best correlated with extractable Ca in the soil. Recent work[26] suggests that soil pH, *per se*, is more important than soil Ca level in influencing cast production by *A. caliginosa*. This is illustrated in Fig. 4 where cast production increased with Ca additions as $CaCO_3$, up to a certain level. In contrast, cast production was unaffected by increasing levels of Ca, added as $CaSO_4$,

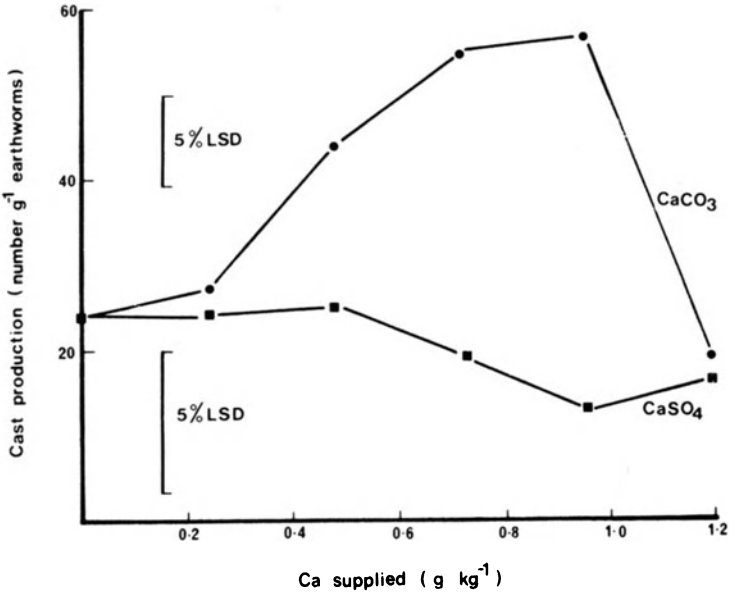

Fig. 4. Cast production of casts (number g^{-1} earthworm) by *A. caliginosa* as influenced by amount of Ca supplied as $CaCO_3$ and $CaSO_4$ to a soil initially at pH 5.9. Data from Springett and Syers[26].

which had essentially no effect on soil pH. It may be anticipated that the initial pH of the soil would have a major influence on the extent to which lime addition influences the activity and species composition of the earthworm fauna.

Drainage might be expected to have a beneficial effect on earthworm activity, although this has been little studied. Earthworm species differ in their ability to survive waterlogged conditions but several beneficial species can apparently survive for several weels submerged in water[11]. Under field conditions waterlogging is associated with low oxygen tensions which may adversely affect earthworm activity. It is of interest that Waters[30] found periods of high soil moisture were usually followed by a decrease in earthworm weights.

Conclusions

Soil fertility, as defined in the present paper, is improved by the activity of earthworms and this can increase the growth of plants. Earthworms are present in most soils but their production and activity may require stimulation by using appropriate management practices, *e.g.* liming, direct drilling, return of organic matter, and more careful use of pesticides. In other situations, it may be necessary to introduce more effective earthworm species.

Two current issues facing soil-related aspects of agriculural production are soil physical limitations to crop growth and the efficiency of fertilizer use. It has been shown that earthworms can have significant positive effects on both these aspects but there is still a need for research on the mechanisms by which earthworms bring about improvements. This is required before suitable management practices can be developed to optimise the physical and biological effects of earthworms on nutrient supply. Lack of specific information on the interaction amongst earthworm species, soil properties, and crop requirements is restricting the concept of soil fertility to one which has a strong chemical basis, operationally defined by analytical chemistry. In some cases, this could lead to the unnecessary use of increasingly-expensive, manufactured fertilizers.

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Effect of lime, phosphorus and mycorrhizal fungi on growth, nodulation and nitrogen fixation by white clover (*Trifolium repens*) grown in UK hill soils

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Key words Hill soils Lime Mycorrhiza Nitrogen fixation Nodulation Phosphorus Rhizobium Symbioses White clover

Summary The responses of white clover (cv NZ Grasslands Huia grown in four UK hill soil types) to additions of lime and P, to inoculation with Rhizobium and mycorrhizal fungi, and to differences in soil water status were assessed in pot and field experiments. With a deep peat soil in pots, shoot production, nodulation and N fixation by clover were increased by 160, 130 and 85% respectively following inoculation with mycorrhiza, but in the field, despite a doubling of root infection, there was no response in growth. On a brown earth soil in the field inoculation with one endophyte (*Glomus mosseae* L1) out of four tested depressed production of white clover shoots by 42% but enhanced that of leeks (*Allium porrum*) by 50%; the others were without effect. With dry peaty podzol and brown earth soils in pots, clover shoot production was highest with added P when a water holding capacity of 80% was maintained, but roots from the latter had only 2.6 compared to 68 nodules per plant from the former. Further work is required to explain poor nodulation in the brown earth soils.

Introduction

Most UK hill soils are acid, high in organic matter and total amounts of N and P but have extremely small amounts of available plant nutrients[6]. Indigenous plants adapted to tolerate these soil conditions and the cool, wet and windy climate of these areas are relatively unproductive and their herbage is of poor nutritional quality; only rarely are native legumes present[12]. To improve herbage production and quality for grazing livestock, whose progeny constitute the main agricultural product of these regions, it is necessary to introduce more productive grasses and also a legume, usually white clover, which adds atmospheric N to the system. The N fixed can be partially transferred to companion plants, and the herbage of white clover is of high value for livestock.

Early experiments at the Organisation and elsewhere indicated that white clover would not grow in hill soils unless they were amended by the application of lime, P and K[10,15] with care to balance the latter two nutrients[7]. A pH of at least 5.2 is required, and preferably 5.5–6.0, to grow well in soil; thus most hill soils have to be limed before the introduction of clover can be contemplated. Lime dressings of 2.5–7.5 t/ha are used, the larger amounts on peaty soils in the wetter parts

of the country and the smaller dressings on more basic mineral soils in the drier east. Consistent response to rhizobial inoculation using the peat inoculum technique was found only in deep peat and wet peaty podzol soils[13] and while responses to mycorrhiza were found in the laboratory they could not be reproduced predictably under field conditions[17].

Some recent experiments on clover microbial interactions are described with emphasis on how applications of lime and P and inoculation with vesicular arbuscular mycorrhizal fungi (VAM) affect growth, nodulation and N fixation by the plants grown mainly in a deep peak soil but also in other UK hill soils.

Materials and methods

Soils

One deep peat, two brown earth and one dry peaty podzol soils were used (Table 1). For pot experiments freshly collected damp soils were sieved to pass a 2 mm mesh; the peat was shredded before sieving. Most probable number techniques[2,15] were used to count the number of rhizobia and VAM propagules in the soils; all had less than 40 rhizobia per g soil which was the lower limit of detection and there were 0.3, 2.5, 2.5 and 5.4 VAM propagules per g soil in Soils 1–4 respectively.

Table 1. Some characteristics of the UK hill soils

	Loss on ignition %	pH in H_2O	Extractable nutrients* mg/100 g dry soil Ca	P	Al
1. Deep peat Lephinmore, Argyll	86	3.8	69	1	15
2. Brown earth Cleish, Fife	18	4.8	161	0.1	45
3. Brown earth Sourhope, Roxburgh	25	4.5	47	6	18
4. Dry peaty podzol Baddinsgill, Peebles	78	4.5	78	3	1

* Nutrients extracted by 0.125 M NH_4 AC buffered to pH 4.5

Rhizobium

A mixture of two strains (P3 and FA6) from the Edinburgh School of Agriculture collection, giving about 10^4 organisms per white clover seed when applied by the peat inoculum technique[20] was used in all the experiments. These strains had been selected for their effectiveness at nodulation and N fixing by Professor A J Holding from clover growing on a deep peat and a brown earth respectively. The number and size of nodules on white clover roots were assessed.

Mycorrhiza

A mixture of indigenous strains with predominantly coarse hyphae, isolated and maintained on maize, were used with Soil 1. Subsequently, three endophytes provided by Dr F E Sanders (Leeds University) – *Glomus clarus*, *Glomus macrocarpus*, and *Glomus mosseae.* and one from Dr M J Daft (Dundee University) *Glomus mosseae* L1 were used in laboratory and field

experiments with Soil 2. The mycorrhizal inoculum was a mixture of spores and infected root segments diluted in sterile sand. At harvest, roots were washed free of soil, cleaned and stained[14] and the level of infection was determined using the grid intersect method[88].

Plant culture

In the glasshouse or growth room 10 cm diameter pots were filled with soil after uniformly mixing with fertilizer and/or lime finely ground to pass a 250 mm sieve; 40 (Soil 1) or 10 (Soils 3 and 4) white clover seeds were sown in each pot. Tricalcium orthophosphate was the form of P fertilizer used in experiments 1 to 3 and superphosphate (21% P_2O_5) in 4 and 5. For experiments with mycorrhiza the inoculum (10 g/pot) was placed in a layer 1 cm below the level of the seeds. In the field at Cleish the soil was limed (5 t/ha), ploughed and rotovated; one week before sowing 100 kg K/ha as KCl and 0 or 50 kg P/ha as granular superphosphate were mixed into the top soil, trace elements were sprayed on to the surface and the white clover seed inoculated with Rhizobium was sown at 5 kg/ha together with the mycorrhizal inoculant; equal volumes (5 m^3/ha) of inoculant or sterile sand were used. Shoot and root dry weight was recorded and the content of P in shoots was determined by a modified Allen's[1] method using Hg as the catalyst. The rate of N fixation was estimated by using the acetylene reduction assay[3].

Results

(a) *Deep peat soil (Soil 1): Experiments 1–3*

1. *Effect of lime* Data are presented in Table 2 from a pot experiment with Soil 1 on which white clover was grown for 203 days, having been cut three times. The pH of the untreated soil was 3.0, and with the equivalent of 5.8 t lime/ha this was raised to pH 7.0; it is very noticeable that the soil pH fell during the course of the experiment. Shoot and root production was enhanced by 1.5 and 2.9 t lime but that of shoots declined at 5.8 t, possibly because the dissolution of the P fertilizer (tricalcium ortho-phosphate) was slower at pH 7.0 and the uptake of P by the plant roots was less, or it was due to excess conductivity in the soil solution or induced trace element deficiency[15]. Nodulation was markedly increased with each lime application though at 5.8 t the number of nodules per g root DM was the same as that at 2.9 t. The total amount of N accumulated in the shoots of white clover during the three growth periods was greatest when 2.9 t lime/ha was applied where the maximum soil pH was 5.5 and where there were 5.2 nodules per plant.

2. *Effects of applied P and inoculation with indigenous VAM fungi* In Soil 1 large yield responses by white clover to applied P are typically obtained in both pot and field experiments[7] and a critical P content of 0.20% of the DM in white clover shoots was derived from pot experiments using this and other hill soil types[15]. The relevance of critical concentrations and their value as a diagnostic for nutrient deficiencies in pastures in the field were discussed by Rangeley and Newbould[17].

Table 2. The effect of lime on soil pH, on production of herbage and root DM (mg/plant) and on nodulation and N in shoots of white clover grown in Soil 1[15]

Lime (t/ha)	pH of soil at sowing	pH of soil at 3rd cut	Shoot DM (Total 3 cuts) mg/plant	Root DM at cut 3 mg/plant	Number of nodules per plant	Number of nodules per mg root DM	N in shoots (Total 3 cuts) mg/plant
0	3.9	3.5	75	30	0	0	1.7
1.5	4.9	4.0	211	51	2.9	0.06	7.1
2.9	5.5	4.5	249	69	5.2	0.10	7.5
5.8	7.0	5.5	200	86	6.3	0.10	4.9
LSD ($P < 0.05$)	0.02	0.04	6.8	5.7	0.56	0.014	0.12

Table 3. The response of white clover, growing in Soil 1 in the growth room, to enhanced infection of the roots with mycorrhizal fungi at two levels of added P

Inoculant	P (kg/ha) 48 −	48 +	160 −	160 +	LSD ($P < 0.05$)
Root infection (%)	13	54	3	47	5.0
Plant DM (mg/pl)					
shoot	18	45	50	58	6.1
root	8	20	18	23	3.2
total	26	65	68	81	8.7
P in shoots (%)	0.29	0.22	0.38	0.41	0.041
Nodules/pl < 1 mm	5.1	9.4	15.9	17.7	2.18
> 1 mm	0.4	3.3	4.8	5.6	1.07
C_2H_2 reduction (n moles/pl/h)	50	475	825	850	164
N in shoots (mg/pl)	1.0	1.8	2.0	2.1	0.25

Table 3 records the effects of applying P and inoculating with VAM on growth, nodulation and N fixation by white clover grown for 47 days in a pot experiment.

Shoot yields were almost trebled and the N content was doubled by increasing the P from 30 to 160 kg P/ha and the number of nodules and the rate of acetylene reduction, measured just before harvest, were all greatly increased. Inoculation with VAM was particularly effective at the lower level of applied P and with shoot P concentrations close to 0.20%; here an increase in root infection from 13 to 54% more than doubled shoot DM and the acetylene reduction rate and almost doubled nodule numbers and the N content of the plants.

3. *Effects of applied P and mineral N, and inoculation with* Glomus mosseae *L.* Table 4 records yields of shoots and acetylene reduction rates with white clover either fertilized with NH_4NO_3 or dependent solely on N fixation. *Glomus mosseae* L1 increased yields after 48 days

Table 4. The response of white clover, grown in Soil 1 which derived N from mineral-N or from N-fixation, to inoculation with *Glomus mosseae* L1 and to applications of P

	P (kg/ha)						
	40			160			LSD
VAM inoculant	−	+	% change	−	+	% change	($P < 0.05$)
Shoot production (mg/pl)							
Fixed-N	18	66	+267	207	194	−6.3	13.5
Mineral-N	24	70	+192	278	264	−5.0	
Acetylene reduction (n moles/pl/h)							
Fixed-N	0	373		2620	2430		161.0
Mineral-N	0	0		0.1	0.1		

growth with 40 but not 160 kg P/ha. With 40 kg P/ha mycorrhizal inoculation increased yield by 267% when the plants were fixing N but only by 191% when the plants were growing on mineral N. This difference may indicate interaction between the rhizobia and the mycorrhizal fungi other than an indirect effect of P uptake on plant growth and photosynthesis with consequent carbohydrate available for the nodules.

The beneficial effects of inoculation of white clover with VAM in glasshouse experiments led to an attempt to inoculate white clover with *Glomus mosseae* L1 in a field experiment. The results of this and other experiments are described elsewhere[16] but, in summary, when harvested one year after sowing inoculation had doubled the level of root infection but had no effect on production. The difference in response between laboratory and field experiments was attributed to the lower temperature and greater wetness of the field soil and poor contact between root and inoculum in this uncultivatable soil. It might also reflect differences in the contact between roots and P fertilizer since in the field it was only possible to apply the superphosphate granules to the soil surface.

(b) *Other soils: Experiments 4 and 5.*

4. *Effect of innoculation with VAM fungi on a brown earth soil (Soil 2)* White clover was inoculated with four different types of VAM in the field in Soil 2 which was well drained and cultivatable. Seeds and inoculum were mixed and drilled together into the soil (4 drills per 2 x 4 m plot) to bring the young root of the white clover seedling into immediate contact with the inoculum. The clover responded to added P so a response to inoculation was a depression in herbage DM with *Glomus mosseae* L1, but this was significant only in the presence of added P; the other inoculants were without effect (Table 5).

Table 5. The effect of four mycorrhizal inoculants and superphosphate on the dry weight of shoots and root infection with mycorrhiza of white clover and leeks grown in Soil 2 in the field (124 days)

Plant species		Inoculant	Herbage production (DM)	Root infection (%)
			kg/ha	
White clover	0	None	39	70
		G mosseae L1	21	53
		G Mosseae	37	57
		G macrocarpus	48	73
		G clarus	42	61
	50	None	98	65
		G mosseae L1	57	77
		G mosseae	94	64
		G macrocarpus	100	79
		G clarus	102	77
LSD ($P < 0.05$)			21.5	30.3
			g/plant	
Leek	0	None	14.9	46.1
		G mosseae L1	22.1	78.3
LSD ($P < 0.05$)			6.2	19.8

Adjacent to the clover experiments, leeks (*Allium porrum*) planted 25 cm apart in 50 cm wide rows were inoculated with *G. mosseae* L1 only; by contrast with white clover inoculation increased leek yield. The difference in response by the two plant species may be because leeks have very coarse unbranched roots without hairs and may be more dependent on VAM infection for P uptake than white clover, and/or because the leeks were spaced out more than the clover. Work by Daft (personal communication) indicates that spacing of plants can greatly affect the mycorrhizal response. Alternatively, this result may indicate differences in the symbiotic efficiency of indigenous and/or inoculated VAM with host plant.

5. *Effect of P and soil water status in a brown earth (Soil 3) and dry peaty podzol (Soil 4)* In the absence of indigenous rhizobia it might be anticipated when hill soils are ameliorated to meet the lime and nutrient requirements of white clover that the inoculation of the seeds with Rhizobium would elicit a response in nodulation, N fixation and growth of white clover. In recent field trials this was true only on deep peat or wet peaty podzol soils[13]. The average proportion of nodulated plants found in the sowing year for 21 trials was 74%. Two of these soils (3 and 4 in Table 1) with 68 and 21% nodulated plants respectively were used to investigate the effects of soil moisture (watered twice a day to 40 or 80% water holding capacity) and superphosphate

(20 kg P/ha as in the field trials) applied as granules to the soil surface (Table 6). This design was adopted since it was thought the low nodulation found in the field trials with these soils might have been due to a lack of P when low rainfall prevented dissolution of fertilizer granules and the absence or ineffectiveness of indigenous mycorrhiza.

All plants in Soil 4 were nodulated and nodule numbers, % P in shoot and dry matter yield were generally increased by applying P and by increasing the soil moisture status. Further increases in production with additional P would be expected since the % P in shoots is well below the critical concentration[17]. In Soil 3 the greater moisture content of the soil did increase the proportion of nodulated plants from an average 50% to 81% but the number of nodules on each plant averaged < 3 compared with > 25 for Soil 4. There is no evidence to suggest that this difference in nodule numbers was caused by more mineralisation of N in Soil 3 than Soil 4 because there was, on the whole, less N in shoots of plants grown in the former soil. The pH of the soil was 5.8 – well above that at which soluble aluminium in this soil[19] is likely to interfere with nodulation[11]. Cooper *et al.*[4] in Northern Ireland have shown the importance of aluminium at low pHs in hill soils. While toxic effects of aluminium cannot be entirely ruled out, there must be some other factor inhibiting nodulation which is as yet unknown.

Discussion

From the data briefly reviewed and presented here it is evident that white clover requires a soil pH of about 5.5 and is often short of P in hill soils; thus it usually responds to lime and P fertilizers. It is also clear that responses of white clover to inoculation with rhizobia and mycorrhizal fungi when grown in hill soils under laboratory conditions are difficult to attain in the field. When responses to VAM inoculation do occur our data support the widely-held view[9,18] that the beneficial effect probably lies in an enhanced supply of P for the host, followed by direct or indirect effects on nodule growth and function. Alternative effects on supply of trace elements, N or water or the cross transfer of hormones or other metabolites between fungus and host cannot be entirely excluded; evidence of a possible direct interaction between rhizobia and VAM (Table 4) may indicate one or other of these processes. These data were from the only experiment in the present series where effective rhizobia were not present with VAM so that only partial conclusions about their inter-relationship can be drawn. Much more needs to be known about the distribution and life cycle of

Table 6. The effect of soil water status (40 or 80% water holding capacity) and amount of P (0 and 20 kg P/ha applied as granules to the surface) on herbage production (mg DM/pl), nodulation, amount of N, and concentration of P in shoots (%) of white clover in two hill soils after 60 days' growth

	Dry peaty podzol (Soil 4)					Brown earth (Soil 3)				
	P0		P20		LSD	P0		P20		LSD
WHC (%)	40	80	40	80	($P < 0.05$)	40	80	40	80	($P < 0.05$)
Shoot DM (mg/pl)	62	212	81	430	46.0	42	97	55	89	38.4
Nodulated plant (%)	100	100	100	100	–	41	73	58	90	22.7
Nodules/pl										
< 1 mm	24	43	59	58	26.8	1.3	0	0	0.6	–
> 1 mm	1.3	2.8	4.3	10.0	1.9	0.2	2.1	1.8	2.0	0.8
P in shoots (%)	0.09	0.12	0.15	0.18	0.02	0.16	0.16	0.15	0.15	0.05
N in shoots (mg/pl)	1.5	4.1	2.2	10.9	0.94	0.9	4.9	1.0	5.0	1.18

indigenous endophytes and their adaptivity to changes in soil fertility during hill land improvement before responses to inoculation can be made more predictable and consistent. An endophyte which is a rapid coloniser and highly effective in transporting P to the host is required to ensure the speedy establishment of improved hill pastures and to enhance their subsequent early and late season production. It is encouraging that the results in Table 5 indicate some variability between endophytes.

The maximum total number of nodules found per nodulated plant varied from 68 in Soil 4, 23 in Soil 1 to 2.6 in Soil 3; the use of large nodules only, or expression per unit weight of root, reduces the spread of values but still leaves plants grown in Soil 3 (a brown earth) with few nodules. Moreover, there were fewer nodulated plants on this soil (Table 6). The data presented here throw little new light on the reasons for this and whether soil, plant or microbial factors are responsible.

Irrespective of strain, it appears that the success of inoculation by both rhizobia and mycorrhizal fungi depends on the presence of large amounts of clover root and agriculturally practical methods of inoculation will be required to maximize the impact of microbial symbioses on the establishment and growth of white clover in improved hill pastures.

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Effect of liming on spore germination, germ tube growth and root colonization by vesicular-arbuscular mycorrhizal fungi*

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Key words Al toxicity Fungistasis Liming Mycorrhizal fungi Spore germination VAM

Summary The effect of soil acidity on spore germination, germ tube growth and root colonization of vesicular-arbuscular mycorrhizal (VAM) fungi was examined using a Florida Ultisol. Soil samples were treated with 0, 4, 8 and 12 meq $Ca/MgCO_3$/100 g soil and each lime level received 0, 240, and 720 ppm P as superphosphate. Corn (*Zea mays* L.) was planted in the soil treatments, inoculated with either *Glomus mosseae* or *Gigaspora margarita* spores and grown for 31 days. Acid soil inhibits mycorrhizal formation by *G. mosseae* through its strong fungistatic effect against the spores. The dolomitic lime increased mycorrhizal formation by both fungal species. *G. margarita* is much less sensitive to acidic conditions than *G. mosseae.* Al ions are a very important component of the fungistatic property against the VAM symbiosis. VAM fungus adaptation may be important for plants growing on infertile acid soils if soil inoculation with these fungi is to contribute significantly to low-input technology for tropical agricultural systems.

Introduction

A possible alternative mechanism for maximizing fertilizer efficiency is via inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi. They enhance nutrient uptake, and consequently plant growth, through an extensive network of external mycelium which acts as an extension of the root absorption system[17]. This is particularly important in infertile soils, commonly found in tropical regions, and appears to be a promising technology for subsistence farmers[23]. If economical techniques for inoculation or manipulation of the native population of VAM fungi are to be developed, the adaptability of the fungi to edaphic factors must be considered. Soil acidity is of immediate concern since it is known to affect spore distribution, root colonization and mycorrhizal efficiency[4,18,19]. However, it is not clear if this results from direct activity of the ionic concentration of H^+ and/or from changes in the chemical properties of the soil. Hubbell[12] suggests that liming may influence the development of mycorrhizal associations either by decreasing the growth of fungal propagules in the rhizosphere or by decreasing fungal colonization of root tissue.

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Germination of VAM fungal spores on either agar media or soil is affected by the pH[8]. However, soil acidity is not an independent factor; pH alone may have little significance in understanding fungal spore germination and root colonization by these fungi. We have recently found significant interactions between pH and medium composition for spore germination "*in vitro*"[25]. This suggests that the effect of soil acidity on VAM fungi may be the result of changes in solubility of nutrients and toxic elements in the soil environment, rather than pH "*per se*".

This study attempts to evaluate the effect of soil acidity on spore germination, germ tube growth and colonization of corn root by two VAM fungi.

Materials and methods

Samples of a virgin Dothan fine sandy loam (thermic Plinthic Paleudult) soil were collected at 0–20 cm depth from an area under a 20-year-old stand of slash pine in Escambia County, Florida. The samples received the equivalent of 0, 4, 8 and 12 meq $Ca/MgCO_3$/100 g of soil (as dolomitic limestone containing 54% $CaCO_3$ and 43% $MgCO_3$). After incubation for 3 months with the water content maintained at 22% by weight, 0, 240, and 720 ppm of P as superphosphate (46% P_2O_5) was applied simultaneously with 200 ppm of K as KCl and 20 ppm of trace elements (frit) as FTE 503 (18% Fe, 7.5% Mn, 7.0% Zn, 3% B and 0.2% Mo). The samples were again incubated for 3 months, then air dried and stored in plastic bags until used.

For the greenhouse experiments, 250 g of soil was packed into styrofoam cups. Fifty spores per cup of either *Glomus mosseae* or *Gigaspora margarita* were applied 3 cm below the soil surface, and 25% w/w water was added. This was a factorial experiment composed of 4 lime treatments × 3 P levels × 3 VAM treatments × 3 replications. Three imbibed seeds of corn (*Zea mays* L. var. Dekalb) were planted per pot and thinned to one plant five days after emergence. After growing for 31 days under greenhouse conditions, plants were harvested and individual root samples were cleared and stained[20] for root colonization assessment using the grid intersect method.

At the same time, soil samples with no applied P and different limestone treatments were brought to the laboratory for spore germination studies. Twenty spores of either *G. mosseae* or *G. margarita* were placed between two Gelman membrane filters (.45 μm) and incubated between two 50 g layers of soil in 100 × 15 mm Petri dishes. Each limestone treatment was triplicated. Washed river sand was used as control. Soil moisture was adjusted to 25% w/w, the plates were wrapped with aluminium foil and incubated at 28°C for 20 days. After incubation, filters with spores were removed from the plates and flooded with either trypan blue or acid fuchsin (both 0.01%) staining solution for 5 min. Following staining, spores were observed under a dissecting microscope at ×12 or 25 and spore germination and germ tube growth rate were recorded according to Siqueira *et al.*[25].

Water extracts from the different soil samples were obtained using 100 g/100 ml of deionized water, shaken for 48 hours and prepared according to Ko and Hora[13]. These were incorporated in agar plates for studies of their effects on spore germination and growth features. Experimental details are given by Siqueira[24] and Siqueira *et al.*[25] but only data for germination are included here (Fig. 2).

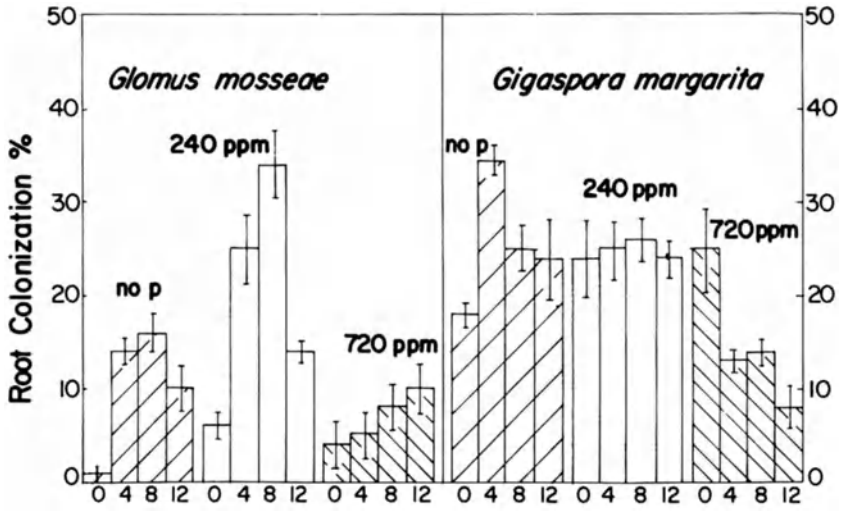

Fig. 1. Effect of liming (0.4, 8 and 12 meq $CaMgCO_3$/100 g soil) and P fertilization (0, 240 and 720 ppm) on VAM formation on corn roots growing in a Ultisol infested with VAM fungi. Bars represent standard deviation for 3 replications.

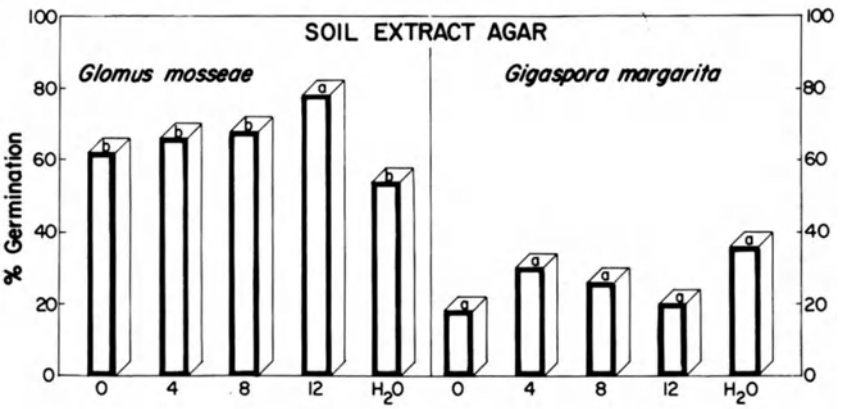

Fig. 2. Effect of water extracts from soils incubated with different levels of lime (0, 4, 8 and 12 meq $CaMgCO_3$/100 g soil) on spore germination of two VAM fungal species "*in vitro*".

Results and discussion

Root colonization

Addition of dolomitic limestone to the acid soil increased root colonization by the introduced VAM fungi except for *G. margarita*, at the highest level of applied P (Fig. 1). Colonization by the indigenous mycorrhizal fungi reached a maximum of 7% and was not affected by either lime or P applications (data not shown). The mycorrhizal inoculum potential, as determined by the most probable numbers method

Table 1. Water pH and 0.5 *N* H_2SO_4 + 0.025 *N* HCl soluble elements (ppm) for the different soil samples with no P application, and their relationship with spore germination, germ tube growth, and root colonization by *G. mosseae*

Lime level (meq/100 g)	pH (H_2O)	Al	Ca	Mg	P	K	Mn	Fe	Zn	Cu
Sand	7.0	4	13	4	10	1	0	19	0.92	0.08
0	5.5	772	26	12	3	29	0.42	0.72	1.08	0.16
4	5.9	766	382	222	3	16	0.92	0.92	0.68	0.16
8	6.1	746	702	378	3	14	1.14	1.14	0.56	0.12
12	6.4	652	1120	550	3	11	1.44	1.44	0.64	0.12
Correlation coefficients										
Germination	0.97*	− 0.83*	0.07	0.05	0.18	0.68*	0.22	− 0.79	− 0.27	− 0.93*
GT growth	0.72	− 0.48*	0.31	0.30	0.08	− 0.78*	0.09	− 0.49	− 0.56	− 0.81*
% Colonization	0.63	− 0.13	0.09	0.60	0.67	− 0.82	0.67	− 0.12	− 0.95*	− 0.48

Soil organic matter = 3.7%; Al saturation = 68%.
* $P \leq 0.05$.
No significant relationship was found between these parameters for *G. margarita.*

on fresh soil samples, was 47 propagules per 100 g soil, and the native spore population was dominated by *Gigaspora calospora, Acaulospora* spp., and *Entrophospora* spp.[15]. The low infectivity exhibited by the indigenous mycorrhizal fungi may be the result of decreased viability caused by the long term storage and incubation (9 months) of the soil without the host plant. As already reported[4,27] *G. margarita* was less affected by liming and showed greater colonization rates than *G. mosseae.* These results suggest that *G. margarita* is more adapted to low-P acid soils than *G. mosseae,* which does not establish well in acid soils, sometimes even after liming. Soil pH and chemical elements extracted by 0.5 *N* HCl + 0.025 *N* H_2SO_4 (double acid) did not explain the variability in root colonization except for a significant correlation ($P = 0.05$) found for Al, Zn and root colonization by *G. mosseae* (Table 1). Yawney *et al.*[27] also reported no significant correlations between soil pH adjustment and percent root colonization on sweetgum plants inoculated with *G. margarita.* McIlveen and Cole[16] showed that addition of a small amount of $ZnSO_4$ enhanced soybean root colonization by *G. mosseae,* while rates higher than 45 mg Zn/kg soil resulted in decreased colonization. The low root colonization by *G. mosseae* in soil with no limestone could be due to Zn and Al toxicity caused by their higher solubility under acid conditions (Table 1). The relationship of these elements to spore germination will be discussed in a later section of this paper. Different mechanisms may operate at different H^+ ion concentrations; thus, for the unlimed

soil root colonization may be affected by high Al and Zn concentrations, while at high lime rates the high basic cations and low available Mn could be the operative factors (see Table 1). Elmes *et al.*[5], using a nutrient film technique, found that Ca at 75 mg/l gave better corn root colonization than at 15 or 150 mg/l: they indicated tht the highest level was approaching toxicity. Soils that received high rates of lime had Ca levels high enough to reduce spore germination[24].

VAM fungi have been reported to occur in plants growing in bituminous mine spoil at pH 2.7[3] and in alkaline soils at pH 9.2[2]. Sparling and Tinker[26] found no effect of pH on root colonization in grassland sites of pH 4.9, 5.9 and 6.2. These observations support the concept that pH alone is not the controlling factor for root colonization in acid soils. However, roots from these sites may be colonized by strains of different fungal species tolerant of heavy metals[6]. Indeed, *Glomus* species and isolates differed in their ability to form mycorrhizae at low pH[14]. The lack of correlation between soil chemical properties and root colonization, also found by Yawney *et al.*[27], suggests that the effect of soil acidity on the VAM symbiosis is very complex and it is more likely to operate on the fungus than on the host plant. Soil acidity may reduce not only mycorrhizal formation, but also the production of VAM fungal spores and their viability in natural soils[21]. Due to Al and Mn toxicities and low P in soil solution most crop species growing in acid soils have coarse, stunted root systems confined to the top soil layer. Because of their beneficial effect on P uptake and plant-water relationships, VAM associations would play a very important role in crop production under acid soil conditions if both host and fungus species could be selected for adaptation to such an environment[24]. Sanchez and Salinas[23] have speculated that the ability to form VAM associations may be important for crop species and varieties adaptable to low-input systems. Depression of plant growth by over-liming highly weathered tropical soils has been attributed to reduced P uptake and induced trace element deficiency[22]. However, our results and those of Yawney *et al.*[27] show that applications of high levels of limestone can depress root colonization by *G. mosseae* and *G. margarita*. This factor may be important since VAM fungi normally increase P and trace element uptake by the host plant[17].

Soil incubation

The effect of soil acidity on spore germination, germ tube (GT) growth and theoretical mycorrhizal colonization index (MCI = % germination x GT growth rating) are presented in Table 2: additions of limestone enhanced all three indices. The germination of *G. mosseae*

Table 2. Spore germination percent (%G), mean germ tube growth rating spore (GT) and theoretical mycorrhizal colonization index (MCI) by VAM fungi incubated for 20 days on acid soil treated with different limestone levels

Limestone level meq/100 g	*Glomus mosseae*			*Gigaspora margarita*		
	%G	GT	MCI	%G	GT	MCI
0	43c	0.9d	39d	50b	3.8b	190b
4	60b	1.8c	108c	50b	4.3ab	215b
8	68b	3.8a	259a	63a	4.7a	296a
12	67b	2.5b	168b	38c	4.4ab	168b
sand	88a	3.4a	299a	39c	3.0c	117c

MCI = %G x GT growth rating.
Means followed by the same letter are not statistically different by Duncan's test at 0.05 level.

was increased above that of unlimed soil by as much as 58%, but still remained below the control (sand), while the maximum increase for *G. margarita* was 26%, in both cases with 8 meq $Ca/MgCO_3$/100 g of soil. Germination rates and GT growth for *G. margarita* were higher than the control treatment (sand) except for the highest level of liming. In general, *G. mosseae* spores that germinated in unlimed soil had stunted GT; liming increased their GT growth by as much as 320%, while in *G. margarita* maximum increase was about 24%. These data suggest that acid soil is fungistatic for VAM fungal spores and that this fungistasis is far more active on *G. mosseae* than on *G. margarita*, which apparently has adapted to the acid environment. Correlation analysis showed significant ($P \leq .05$) relationships of *G. mosseae*, spore germination with pH and K(+), Al and Cu(−); while the GT growth correlated negatively with K and Cu (Table 1). For *G. margarita* spores no significant relationship was found between either spore germination or GT growth and soil chemical properties. This suggests the germination process and GT growth in *G. mosseae* is more sensitive to chemical factors under acidic conditions. In fact, *G. mosseae* does not form GT on agar medium with pH adjusted to 4.5 with 1*M* HCl (Siqueira, unpublished); shows very low germination rates at pH 5.0[8]. and is not usually found in soils with pH below 5.0[1]. The toxicity of Cu ions to VAM fungal spores has been demonstrated by Hepper[9]. Amendment with Cu at 0.5 mg/l reduced the germination of *Glomus caledonicum* to 16% of the control[10].

The results in Table 3 indicate that the inhibitory effect of unlimed soil on *G. mosseae* spore germination was not removed by the presence of the host plant, by addition of root exudates, or by autoclaving (Table 3).

Volatile compounds produced by soil microorganisms can be either fungistatic or stimulatory for spore germination and GT growth.

Table 3. Effect of different treatments and experimental conditions on germination and GT growth by *G. mosseae* after 12 days of incubation. Means for 3 replications ± standard deviation

Treatments	% Germination	GT Growth rating
Sand control	90 ± 6.4	4.7 ± .60
Unlimed soil	31 ± 5.1	.5 ± .31
4 meq/100 g $CaCO_3$ soil	68 ± 9.2	1.5 ± .40
Unlimed; autoclaved soil	17 ± 3.5	.5 ± .63
4 meq/100 g $CaCO_3$; autoclaved soil	44 ± 1.3	1.1 ± .48
Unlimed; transferred[a]	69 ± 12.7	4.6 ± .65
Unlimed; membrane[b]	82 ± 9.2	4.2 ± .36
4 meq/100 g $CaCO_3$; membrane[b]	77 ± 3.5	4.1 ± .19
Unlimed + Plant[c]	19 ± 1.5	.9 ± .14
Unlimed + root exudates[d]	18 ± 1.4	.5 ± .40
Sand + unlimed soil extracts[e]	67 ± 2.1	3.6 ± .21
Volatile; from unlimed soil[f]	69 ± 8.5	4.2 ± .35
Volatile; 4 meq/100 g $CaCO_3$ soil[f]	71 ± 7.7	4.8 ± .21
Volatile; 8 meq/100 g $CaCO_3$ soil[f]	68 ± 18.1	3.9 ± 1.01
Volatile; 12 meq/100 g $CaCO_3$ soil[f]	76 ± 13.2	4.0 ± 1.41
Volatile; H_2O (control)[f]	61 ± 3.8	3.8 ± .91

[a] Spores were incubated on unlimed soil for 5 days, then transferred to washed sand plates.
[b] 50 g of unlimed soil and limed (4 meq $Ca/MgCO_3$/100 g soil) was packed into a dialysis tube (14K cut off) and spores placed outside on MF filter in sand.
[c] Spores were placed to germinate in the rhizosphere of *Phaseolus vulgaris* growing in fungistatic soil (no lime).
[d] Root exudates from *Phaseolus vulgaris* were used to bring soil moisture to an optimum for spore germination on plates with unlimed soil.
[e] Unsterile soil extracts from unlimed soil samples were used to moisten sand plates where spores had been plated to germinate.
[f] Effect of volatile compounds from soil samples incubated with different levels of limestone.

Hora and Baker[11] reported that increasing the pH of acidic soils resulted in the release of volatile compounds inhibitory to two test fungi and stimulatory to three others. Germination of *Acaulospora laevis* spores was induced by soil volatile compounds while germination of *G. margarita* spores was inhibited by volatile compounds from soil actinomycetes (Siqueira and Bleakley, unpublished). The effect of volatile compounds released from soil samples treated with different limestone rates on either spore germination of GT growth of *G. mosseae* chlamydospores (Table 3), cannot account for the differences found in our experiments.

Nature of the inhibitory factor

In the case of *G. mosseae* the results obtained for spore germination and GT growth in the soil (Tables 2 and 3) and soil extract-agar (Fig. 2) suggest the presence of a water-insoluble fungistatic factor, very similar

to that reported for *Neurospora terrasperma* by Ko and Hora[13]. Evidently, Al toxicity is a very important component of the fungistasis complex present in acid mineral soils. Indeed, in other experiments using the same soil, *G. mosseae* spore germination was negatively correlated with exchangeable Al more than with either corn root colonization or shoot dry weight[15]. Soil acidity appears to affect VAM fungal propagules, mostly on the fungus in the rhizosphere before root penetration takes place rather than on the host itself. These results and others those of Hepper[9] and Hepper and Smith[10] indicate that metals such as Al, Zn, Cu, and Mn and H^+ ions present in soil solution may be toxic under acid conditions and may reduce plant growth by reducing spore germination, GT growth, and hence root colonization by the VAM fungi. In acid tolerant host-fungus combinations, metal toxicity may result in poor growth of external mycelium, nutrient uptake by fungal hyphae, and hence non-efficient VAM symbiosis[4,7].

Conclusions

Liming acid soils to reduce H, Al and other metal toxicities reduces the fungistatic effect against some VAM fungi, favours mycorrhizal formation, and plant growth.

Species and isolates of VAM fungi differ in their tolerance to soil acidity. Because VAM symbiosis increases plant nutrient uptake and growth, acid-soil tolerant species of VAM fungi may play an important role in plant resistance to low P in soil solution and high Al saturation found in most tropical soils. Overliming and heavy fertilizer applications of highly weathered soils of the tropics may reduce mycorrhizal formation and depress plant growth.

Soil acidity appears to be an edaphic factor with great influence on the distribution of VAM fungal species and may be a serious constraint for introduction and longevity of VAM fungi as efficient plant growth promoters and biocontrol agents such as *G. mosseae,* into tropical agricultural production systems.

Inoculations under greenhouse and laboratory conditions, and monitoring spore germination and saprophytic growth by these fungi in the soil, may be a useful way to select species and isolates for further inoculation studies.

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Section 3

Measurements of microbial populations and biomass: their roles in soil processes

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Biological and biochemical analysis of soils

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Key words Adenylate pool Biomass volume CO_2 evolution Chitin DNA Electron microscopy Enzymes Fluorescent antibody Fumigation-respiration Fungi Histochemistry Imunofluorescence Jones-Mollison technique Microcosms Monoclonal antibodies Nitrogen Nutrients Oxygen consumption Phosphorus Phytotoxins Plate counts Rhizobium Rhizosphere Sulphur Xenobiotics

Summary There is an immense literature on biological and biochemical analyses of soils. Such analyses have revealed the enormous richness of species in soil and their vast range of metabolic potentials and ecological diversity. Accordingly, the approaches used to investigate the soil biota and its biochemistry usually have to be modified or adapted depending upon the purpose of the investigation.

Studies of micro-organisms in the soil environment are complicated because microbial cells are commonly attached to surfaces where they live side-by-side with other populations in consortia usually containing different morphological and physiological types. Such assemblages of organisms cannot be described quantitatively using cultural techniques, such as plate counts, which underestimate both cell numbers and viable biomass. The development of more powerful observational and staining techniques has improved our knowledge of the diverse morphological and biochemical composition of soil micro-communities. Such findings have been amplified at a grosser level by laboratory studies with multi-component systems (microcosms) to mimic field situations and to assess the range of biochemical potentials of microbial consortia.

But despite notable advances in analytical methods we are still, with a few exceptions, unable to detect or identify those microorganisms which carry out specific biochemical transformations or determine whether particular cells are alive, dormant or dead at the time of observation.

Considerable work has been done to define some of the fundamental ecological attributes of microbial assemblages in soil. Productive work on the metabolic activities of the soil microbiota, especially geochemical transformations of C, N, S and P, has been under way for more than a century. But only in more recent years have more sensitive and reproducible analytical methods become available to measure viable biomass in soil. This will enable some insight to be gained into the role that microbial biomass plays as a labile source and sink for plant nutrients.

Introduction

The task of providing a complete and comprehensive review of the immense literature of biological and biochemical analysis of soils would be an arduous one. Most of the papers describe new methods or variations of existing methods adapted for the purposes of particular investigations of the myriad species in soil and their vast range of metabolic potentials and ecological diversity. My purpose is to show to what extent biological and biochemical investigations of soils can be

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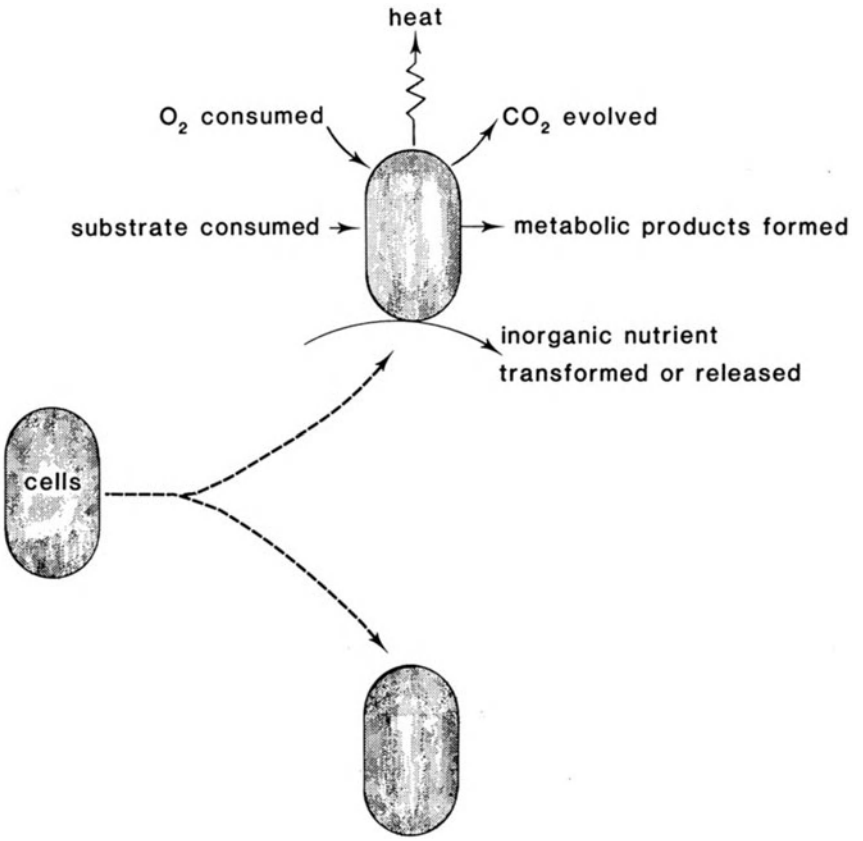

Fig. 1. Indices of microbial activity in soil.

used to provide information about the potential of soils to support the productive growth of plants.

Measures of microbial activity in soil

Fig. 1 attempts to show the major indices of microbial activity of populations of soil micro-organisms. Such indices may also be used to gauge the response of the total soil population to physical, chemical or biological variables. Needless to say various selective methods are

available to measure populations and activities of specific components of the soil populations, *e.g.* nitrifiers or herbicide decomposers. Many of the methods mentioned herein are described and their limitations discussed by Parkinson *et al.*[19] and by the authors of chapters in Rosswall[23] and Burns and Slater[3] and also by Waid[28].

Changes in cell numbers can be measured by direct counting or by viable counting using dilution plate or most probable number (MPN) techniques. Direct counting can also be used to measure total cell volumes. Physiological approaches can be used where rates of consumption or production of chemical substances are measured. Examples include rates of consumption of oxygen in aerobic soils or of alternative electron acceptors, *e.g.* nitrate or sulphate, in anaerobic soils; disappearance of substrates, *e.g.* glucose or cellulose; and transformations of inorganic nutrients, *e.g.* nitrite in the case of Nitrobacter. Products of physiological activity that can be measured include heat, carbon dioxide, organic metabolites, *e.g.* organic acids, and inorganic products, *e.g.* nitrate. There is a range of biochemical components of microbial cells that may be measured, but it is usually difficult to convert the values measured to estimates of biomass. Another more general complication is that the attribute measured is not confined to living cells: it may persist in dead cells or reside in the soil in the absence of living cells *e.g.* chitin, muramic acid, an extracellular hydrolytic enzyme or nucleic acid. However, measurements of total ATP, the total adenylate pool or, following soil fumigation, the total readily-respirable organic carbon have each provided convincing biochemical measures of living cell components which, if used with appropriate caution and experimental controls, can be used to provide seemingly reliable estimates of total biomass in soil.

Biology of soil populations

In order to elucidate the role soil organisms play in soil fertility it is first necessary to understand their biology. But our knowledge of the biology of soil populations is limited severely by the techniques that are available. For example, the identification of strains of soil bacteria or fungi is limited to those that can be isolated on dilution or other media, using selective techniques.

Even if the organism has been isolated from soil, there is no certainty whether, at the time of sampling, it was in an active vegetative state or inactive. If it was 'active', it is unknown what the isolate might have been consuming at the time of sampling. Some efforts have been made to select out from soil specific substrates on which micro-organisms

grow and proliferate, *e.g.*, roots at various stages of decomposition, and then surface sterilise or wash them so that one could on appropriate microbiological media isolate organisms originally present on or within them. Thus it is possible to gain a picture of the order of succession of organisms on a decomposing substrate and of the composition of assemblages of organisms as they develop in soil but it is not possible to judge the relative roles of the various organisms in the decomposition process.

Further insights can be gained by experiments with microcosms, perfused soil columns or chemostats inoculated with soil populations and supplied with known substrates at fixed concentrations. In such experiments it is possible to obtain knowledge of consortia of organisms involved in transformations of inorganic substances *e.g.* sulphur oxidation, or the degredation of recalcitrant organic compounds. But such approaches can all be criticised on the grounds that they are somewhat contrived and artificial and far removed from the field situation. Even so, much can be learned about the interrelationships of different organisms as well as their individual and synergistic potentials to transform or degrade materials.

In recent years considerable advances in methods to observe microorganisms in their soil habitats now provide a powerful battery of techniques, not only to see what organisms are present in soil, but also to locate individual cells of known strains. To a limited extent, it is possible to determine if the organisms are in an active metabolic state and what they are metabolising.

In the field of light microscopy, notable advances have been made in the use of fluorescence, especially epifluorescence. The use of stains such as acridine orange, fluorescein isothiocyanate, europium chelate and ethidium bromide has made it much easier to see the smaller bacteria amongst and on solid soil particles. The cells observed in this way can be counted, thus enabling estimates to be made of the total size of the microbial population in soils. This can be done by a direct counting technique developed by Jones and Mollison[14] or a variation thereof. The stained cells in random fields of soil suspensions are counted and the size of the microbial population per unit volume or weight of soil is estimated. The cell sizes can be measured and their volumes estimated and from these data the total volume of the microbial biomass (biovolume) calculated[10]. It can also be used to determine the biomass of fungi and various categories of bacteria (cocci, rods, filaments).

The use of the Jones and Mollison technique has shown that the total population cells in much larger (about $\times 10^3$) than indicated

by plate culture counts of the same population. This discrepancy arises because many of the cells counted are dead and many of them do not grow, divide and form colonies on the media generally used for plate counting.

The Jones-Mollison direct counting method has formed the basis for a variety of recent investigations of the soil microbial biomass. For example, to obtain a more complete understanding of the soil ecosystem it will be essential to measure the quantities of nutrient elements contained in the biomass and the rates at which they are immobilized and mineralized. To do this, one essential procedural step is to determine the size of the microbial biomass[10]. A direct counting technique can be used as a 'baseline' measurement in the validation of other techniques to estimate the size of the biomass, *e.g.* adenosine triphosphate (ATP) measurement or its metabolic activity[10].

The application of electron microscopes to soil problems has enabled us to see more details of organisms in soil, including their ultrastructure. The scanning electron microscope (SEM) has revealed much about the variety of micro-organisms in soil, where they live and their spatial relationships to soil mineral and organic materials, as well as where they occur on root systems. The SEM has shown that the cells of bacteria may occur in quite large colonies[6]. Indeed Harris[8] was able to show by a soil-film flotation technique that bacterial colonies can be composed of hundreds, even thousands, of uniformly-shaped cells. The transmission electron microscope (TEM) for example, demonstrated the presence on root surfaces of large numbers of bacteria colonising the mucigel where numerous types of micro-colonies can be aggregated together[5].

Information about the morphology and ultrastructure of cells in soil can be amplified by the use of autoradiography to determine which cells are metabolising or accumulating substances labelled with radioisotopes such as tritium or carbon-14 and presumably, if suitable controls are used, living cells can be detected[28]. Histochemical techniques also make it possible to locate enzymes on surfaces of soil bacteria or in organelles of fungal hyphae[16].

Using immunofluorescence techniques it is possible to locate in their soil habitats populations of specific organisms. The organism in question, say an effective strain of Rhizobium, is cultured and then its cells are inoculated into a suitable animal so that a supply of antibody proteins specific to the antigens of the Rhizobium cells can be obtained. The antibody is isolated from the animal's blood and a fluorescent substance, such as Rhodamine B, is attached to it as a fluorescent label. Cells of the specific strain of the Rhizobium in soil can be detected

and located by adding the fluorescent antibody (FA) to soil smears where the labelled antibody will adhere very firmly to the surface antigens of the Rhizobium cells. Excess (unattached) antibody is washed off and the fluorescently-labelled cells can be seen using a fluorescence microscope. The FA technique is not without problems, it is not completely specific, but has proved to be valuable. For example, Reyes and Schmidt[22] have shown that the rhizosphere of a host legume, the soybean, was no more stimulatory to a very successful nodule-forming and effective Rhizobium strain than the rhizosphere of corn. The recent development of the more specific monoclonal antibody technique holds out the prospect that one could trace even more efficiently a particular organism or strain of organism when inoculated into soil or onto plants. It may be also possible to use monoclonal antibodies to detect the presence of specific strains of organisms in the soil habitat.

Soil microbial biomass and nutrient flow

Micro-organisms in soil undoubtedly play key roles in transformations of most of the nutrient elements for plants, in particular, C, N, P and S. In recent years the significance of living soil micro-organisms (the microbial biomass) as sources and sinks for nutrients has been recognised[20]. Considerable effort is under way to develop and use techniques to measure the microbial biomass in soil. Many of the concepts of microbial growth and activity developed from studies in pure cultures, chemostats or microcosms, can, with caution, be applied to the field situation. Difficulties can sometimes arise because so little is known about the interactions of micro-organisms with one another and with their solid environment.

Soil micro-organisms interact closely with soil solids[17] and have very low apparent growth rates. Accordingly, the microbial ecologist has to deal with a very different situation to the classic laboratory culture where fast-growing microbial cells multiply rapidly when suspended in a liquid nutrient medium. Microbial cells in soil also have to survive periods, often of considerable duration, when there is insufficient nutrient to support cell growth and proliferation. To endure they either develop survival structures, *e.g.* spores or cysts, or their cells go into an inactive form in which storage products are used to maintain the integrity of the cells during dormancy. To ensure that the cells have sufficient energy to carry them through periods of nutrient stress, as much as half the organic C available to soil micro-organisms could be devoted to maintenance, with the rest available for growth[20].

Early authors considered that the high populations revealed by direct microscopy included a large proportion of dead cells but recent work with ATP and chloroform-fumigation incubation techniques indicates that most of the cells are dormant and only a small proportion of the population is actively growing. Even so, the active cells appear to be multiplying more slowly than those of strains used in the laboratory. Instead of doubling times of 10 to 60 minutes, cells of soil organisms have been calculated to double once every 40 to 50 hours[7,20].

The ATP content of cells forms the basis for a reliable measure of soil biomass[4,11]. ATP occurs in all living cells and can be measured readily and accurately by the luciferin-luciferase system. Fortunatey ATP in dead cells decomposes rapidly and so does extracellular ATP in soil. The main difficulties in using ATP as a quantitative measure of soil biomass are in extracting it efficiently from soil and in relating the amount of ATP in a soil to its biomass content. Attempts have been made to find other specific biomass constituents that could be used to gauge the biomass in soil. Several have been tried (muramic acid, N-acetyl-glucosamine and the nucleic acid bases) but without success[10].

Chloroform-fumigation incubation has also proved a useful technique to measure soil biomass[12]. The flush of CO_2 caused by $CHCl_3$ treatment is due largely to the decomposition of those organisms killed during incubation. Using ^{14}C-labelled cells added to soil before fumigation, it has been found that the proportion of $^{14}CO_2$ is constant and related to the quantity of biomass C added to the soil. This relationship would be expected to vary with the composition of the active soil population and the presence of any decomposable non-biomass C, but these sources of variation appear to be insignificant. Also, it has been found by several independent groups of investigators that, for many different micro-organisms added to a wide variety of soils, about 41% of the incorporated biomass C is mineralised and respired as CO_2 during incubation[1].

Oades and Jenkinson[18] obtained a close relationship between microbial biomass (determined by the $CHCl_3$-fumigation incubation method) and the ATP contents of a heterogeneous range of 14 Australian soils. They found about 7.5 mg ATP g^{-1} biomass C, a figure that was substantiated by further work with a range of English soils[9]. Jenkinson *et al.*[13] also found close agreement between soil biovolume (measured by direct microscopy) and the flush of respiration caused by fumigation.

With the recently developed techniques for measuring soil microbial biomass it has become possible to relate microbial growth, energy flow and nutrient mobilisation in a number of situations. For example,

Ladd *et al.*[15] studied the decomposition of ^{14}C- and ^{15}N-labelled legume residues, the fate of the C and N, and the formation and decay of the isotope-labelled microbial biomass in four South Australian soils in the field over 4 years. Not surprisingly, the biomass organic residues proved to be less resistant to decomposition than the non-biomass organic residues.

Now that the techniques are becoming available for a better understanding of C, N, P and S flow through the small but labile reservoir formed by the microbial biomass in soil, it has become possible to relate these findings to studies of the chemistry of soil organic matter and the mineral nutrition of plants. Also the microbial ecologist now has much needed information about the growth and dormancy of the large population of micro-organisms in soil.

Soil biological and biochemical processes affecting soil fertility

Table 1 lists a selection of the more important biological and biochemical processes or groups of processes in soil that appear to affect or are related to soil fertility and to plant productivity. In this list items 1 to 13 are, as far as possible, arranged in order of decreasing complexity: from aggregates of multi-step processes, carried out by the total soil biomass or by groups of organisms, to more definable readily-linked several-step processes, such as nitrite oxidation. The latter group of processes have a more obvious and direct influence on soil nutrient status than those at the beginning of the list.

The remaining items (14 to 20) are not so well ordered but include a variety of biological processes that can affect plant growth directly, *e.g.* mutualistic symbiotic associations, or indirectly, *e.g.* formation of soil-binding substances.

It is important to remember that all of these activities of the soil biota take place in an environment where chemical, physical and biological stresses, such as nutrient deficiency, low water potential and predation respectively, may modify or destroy communities of organisms carrying out specific transformations or activities that affect plant productivity. Also, the possibility must be considered that the soil offers an environment where genetic interactions among members of microbial communities through alteration, transfer or exchange of genetic material may lead to the acquisition or evolution of novel metabolic sequences or tolerances, *e.g.* the transfer of genetic factors controlling the formation of enzymes to degrade recalcitrant molecules[25,26] as well as mechanisms for the conservation of genetic information[21]. This is a topic that has not yet received adequate

Table 1. Categories of soil biological and biochemical activities affecting plant productivity

1. Formation and decomposition of soil biomass derived from soil micro-organisms and animals but excluding roots. Organisms may be in an active or inactive (cryptic, dormant) states.
2. Total gross heterotrophic activity of soil biomass
3. Decomposition of recognisable residues of plants, animals or micro-organisms, *e.g.* sloughed root-cortex cells.
4. Decomposition of specific substances, complexes or mixtures formed by plants, animals or micro-organisms, *e.g.* cellulose, lignin, tannins; keratin, chitin, urea; lignified wood, tanned proteins, melanised fungal walls; mucigel, root exudates.
5. Communities (consortia) formed from assemblages of biochemically- or nutritionally-interdependent strains of species to effect degradations or other transformations, *e.g.* metabolism of recalcitrant molecules such as cellulose or lignin, sequential oxidation of $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$ by nitrifying bacteria.
6. Formation, secretion and persistance of active extra-cellular hydrolytic enzymes.
7. Mineralisation of organic compounds containing N, P or S and release of CO_2.
8. Nitrification. Autotrophic oxidation of NH_4^+ in aerated zones of soils.
9. Denitrification. Reduction of NO_3^- by micro-organisms using NO_3^- as an alternate electron acceptor to O_2 in anaerobic zones of soil.
10. Ammonia formation and volatilisation. Occurs when N-rich residues degrade under alkaline conditions, *e.g.* legume leaves, urine patches or when high rates of certain N fertilizers are applied, *e.g.* urea.
11. Non-symbiotic nitrogen fixation by various aerobic and anaerobic bacteria, photosynthetic bacteria, and cyanobacteria.
12. Oxidation of inorganic sulphur compounds, with increase in soil acidity and sulphate concentrations.
13. Formation of reduced inorganic and organic compounds, *e.g.* H_2S, N_2, CH_4, organic acids, H_2.
14. Formation, accumulation and destruction (detoxication) of naturally-formed inhibitory or toxic substances.
15. Formation of substances by rhizosphere and rhizoplane organisms which increase root cell permeability and cause the leakage of organic substances into the root zone.
16. Decomposition of xenobiotics.
17. Synthesis and destruction of humic components of soil.
18. Burrowing by soil animals and formation of root channels.
19. Formation or degradation of soil-binding substances.
20. Symbiotic organisms forming mutualistic or antagonistic associations with plant roots.

investigation in terms of natural mechanisms to modify soil fertility or the possible improvement of soil fertility by deliberate means, *e.g.* introduction of genetically-engineered micro-organisms.

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Extractable N and P in relation to microbial biomass in UK acid organic soils

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Key words Chloroform-fumigation Microbial biomass Mor humus Nitrogen Peat Phosphorus

Summary Samples of peat and mor humus were treated with $CHCl_3$ to kill microbial cells and the amounts of C as CO_2, N as soluble- and mineral-N, and P as inorganic-P released by the treatment were compared with estimates of the microbial biomass by the Anderson and Domsch test and ATP determination. Increased amounts of soluble-N and inorganic-P, extracted with 1 *M* KCl and 0.01 *M* $CaCl_2$ respectively, were detected immediately after the fumigation treatment. However, the subsequent rates of production of CO_2-C and mineral-N measured during a 10-day incubation period at 25°C were low and variable, resulting in anomalously low estimates of microbial biomass. The flush of inorganic-P was more consistent and, in mor humus, generally related to biomass-C as measured by the Anderson and Domsch test.

Introduction

Peats and mor humus are generally acid and low in available nutrients, although the total contents of N are comparatively high[16]. Decomposition of these highly organic soils entails nutrient immobilization in the microbial biomass[17]. The size and turnover rate of the microbial biomass are then significant factors influencing the availability of N and P for plant uptake[2].

Various methods have been used to estimate the microbial biomass in soils but some have proved unreliable in acid conditions. In particular, the production of CO_2-C and mineral-N from decomposing cellular material killed by fumigation with $CHCl_3$ is sometimes anomalously low compared with that in near-neutral soils[11]. In contrast, biomass-P is rapidly transformed by the action of soil enzymes to inorganic-P which is detected immediately after fumigation and the process appears to be less sensitive to low soil pH[5]. We have studied, therefore, the effect of chloroform fumigation on release of CO_2-C, soluble- and mineral-N and inorganic-P in various peats and mor humus. Results were compared with the microbial biomass as estimated both by the Anderson and Domsch test and by measurement of ATP content. The effects of the duration and the method of chloroform treatment have also been investigated.

Materials and methods

Sampling

Details of the sampling sites and some characteristics of the samples are presented in Table 1. Samples were taken from sites 1, 2 and 3 on three occasions during 1980–1982, designated a, b and c (Table 3). The samples of F + H material were collected at three or more areas from the organic horizons beneath the fresh litter layer, bulked and then screened through a 7 mm sieve to remove macrofauna, roots, twigs, cones and stones prior to storage at 4°C. Samples of peat were cut from the surface 150 mm and, just prior to use, thoroughly mixed and live roots removed.

Methods

ATP-analysis ATP content was determined by a modification of the method of Jenkinson and Oades[13] using Sigma FLE-50 luciferin-luciferase enzyme[19]. Average coefficient of variation between replicate samples over a range of organic soils was 19.4 per cent. ATP contents were converted to biomass-C using a conversion factor of 138[11].

Chloroform fumigation Fumigation for up to 18 hr was carried out using EtOH-free $CHCl_3$ [14], the vapour being removed by repeated evacuation. CO_2 evolved from samples subsequently incubated at 25°C for up to 14 days was measured at intervals by gas chromatography[18]. Biomass-C was calculated from the difference in the amount of CO_2-C released between fumigated samples and controls (CO_2-C flush, F_c) using a conversion factor (k_c) of 0.45[11]. The corresponding flush of mineral-N after incubation for 10–14 days (F_n) was converted to biomass-C using a factor of 8[3]. The approximate amounts of N and P in the biomass were calculated from F_n and F_p assuming values of 0.4 for k_n and k_p (ref.[5,12]). Biomass-P was calculated from F_p values measured immediately after fumigation.

Anderson and Domsch test The C content of the microbial biomass was calculated from the rate of CO_2 respiration, measured by gas-chromatography[18] during 30–180 mins at 22° ± 1°C after amending samples with glucose[1]. Preliminary tests showed that finely ground glucose added at rates of 5–10 mg per g fresh weight was adequate for substrate saturation.

Chemical analyses

Soluble organic N together with mineral-N ($NH_4^+ + NO_2^- + NO_3^-$) was extracted with 1 *M* KCl[4]. NH_4^+ in extracts was determined by colorimetric analysis[7] and NO_3^- was measured colorimetrically as NO_2^- after reduction with copperized cadmium[10]. NO_2^-, seldom found in acid organic soils, was not determined separately. Soluble organic-N and NH_4^+-N in 1 *M* KCl extracts was measured as NH_4^+-N after digesting 5 ml aliquots with conc. H_2SO_4 containing 0.1% (w/v) Se[6]. This procedure did not recover NO_3^-. Inorganic-P was extracted by shaking 10 g fresh sample overnight with 50 ml 0.01 *M* $CaCl_2$ and estimated colorimetrically[15].

Moisture content was determined by drying at 70°C and loss on ignition by heating milled, dry samples overnight in an electric muffle at 500°C. For total N and P, dry milled sample was digested with a 1:1 mixture of conc. H_2SO_4 containing 0.1% (w/v) Se and 30% (w/v) H_2O_2 (ref[20]). N and P concentrations in the acid digests were determined colorimetrically[6,15].

Results

Treatment of samples of a larch forest humus (Site 2b) and a raised bog peat (Site 3c) with $CHCl_3$, as a vapour and as a saturated aqueous solution, had a marked effect on the release of mineral-N and inorganic-P (Table 2): the former continued to increase but the latter

Table 1. Site and sample characteristics

Site	Location	Grid Ref.	Soil	Vegetation	Total contents % dry wt N	P	C:N	Loss on ignition %	Moisture % fresh wt	pH (0.01 M $CaCl_2$)
1.	Forest of Glen Tanar	NO516963	F-H layer	110-yr-old Scots pine	1.24	0.211	39	96.3	75.5	2.7
2.	Forest of Glen Tanar	NO503965	F-H layer	25-yr-old larch	0.85	0.178	29	48.7	66.3	3.4
3.	Lyne of Skene	NO765113	Cut-over Raised bog	Calluna Eriophorum Sphagnum spp.	0.71	0.092	68	97.0	72.5	2.7
4.	Lon Mor	NH332072	Blanket bog	Calluna Trichophorum Molinia	2.14	0.062	22	96.0	77.9	3.1
5.	Kershope	NY551808	Flushed Blanket bog	Molinia	2.24	0.162	21	93.2	75.7	3.0
6.	Wanton Wells	NJ624275	Mineral soil Insch series*	Arable-Barley	0.47	ND	12	9.52	25.2	5.3

*Glentworth and Muir[8]
ND Not determined

Table 2. Release of CO_2-C, mineral-N and inorganic-P from samples of a larch humus and a raised bog peat treated with $CHCl_3$ in solution and as a vapour. Samples extracted immediately after treatment or after incubation at 25°C for 14 and 28 days

	CO_2-C after 14 days g kg^{-1} d.wt	Mineral-N, mg kg^{-1} days			Inorganic-P, mg kg^{-1} days		
		0	14	28	0	14	28
LARCH HUMUS							
Control	4.10	83 ± 2	300 ± 13	423 ± 72	1 ± 1	< 0.1	1 ± 1
Vapour	3.96	150 ± 16	759 ± 41	915 ± 21	155 ± 9	310 ± 6	136 ± 13
Solution	3.42	126 ± 20	ND	ND	201 ± 8	ND	ND
RAISED-BOG							
Control	1.78	31 ± 1	65 ± 4	124 ± 22	< 0.1	< 0.1	< 0.1
Vapour	2.15	63 ± 3	260 ± 14	353 ± 37	51 ± 8	66 ± 5	25 ± 3
Solution	1.45	80 ± 9	ND	ND	63 ± 10	ND	ND

1. Average CV for CO_2-C analysis was 8.7 per cent.
2. Mineral-N and inorganic-P values means of 3 replicates ± S.D.

ND = Not determined.

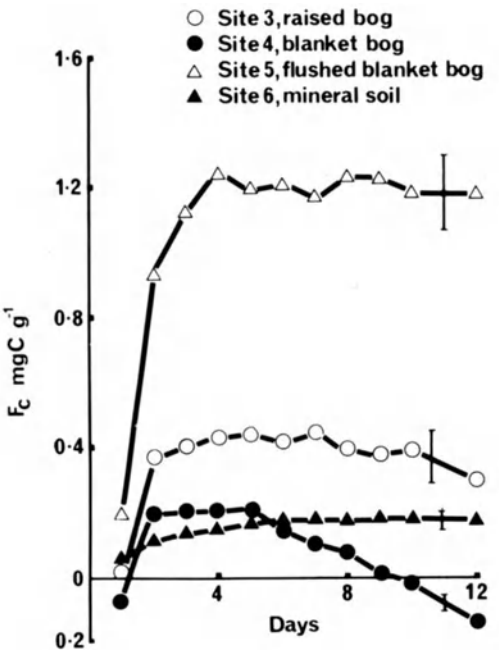

Fig. 1. Changes in values of F_c during incubation at 25°C of control and fumigated samples of 3 peats and a mineral soil. Bars denote average SD.

decreased after incubation for 28 days. There was comparatively little difference between samples treated with either the vapour or the saturated aqueous solution and because the vapour was easily removed, this method was used in all subsequent studies.

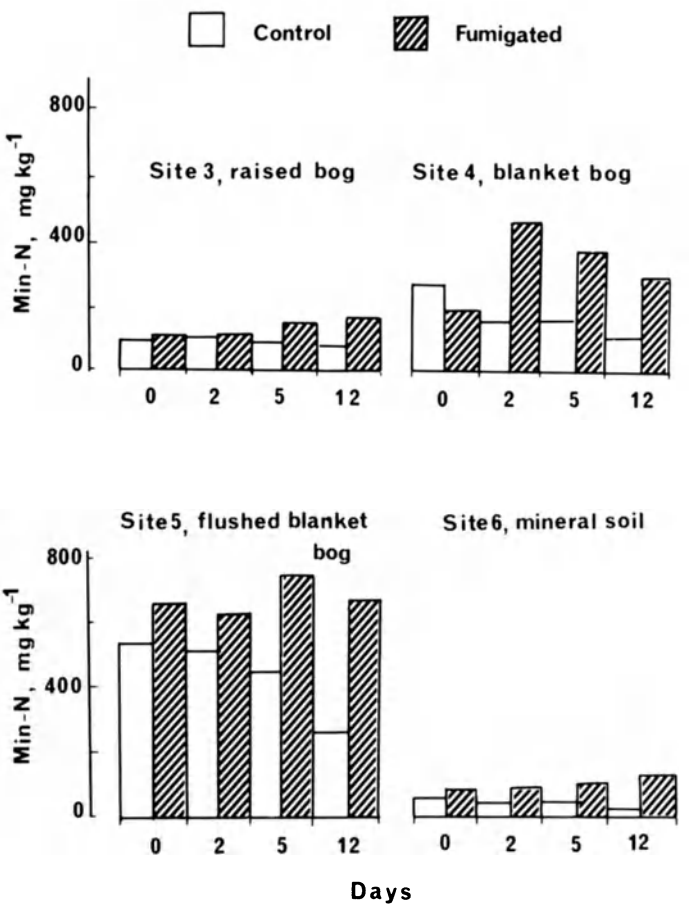

Fig. 2. Mineral-N levels in untreated and fumigated samples of 3 peats and a mineral soil during incubation at 25°C. Average CV between duplicates was 6.0 per cent.

The patterns of release of C as CO_2, N as mineral-N and P as inorganic-P following incubation were observed in samples of three peats and a mineral soil (Sites 3a, 4, 5 and 6).

Fig. 1 shows the C flushes: in the three peats, F_c reached a maximum after 2–5 days and then declined to varying degrees but in the mineral soil, it attained a plateau at about 9 days.

For mineral-N, Fig. 2 shows that the amounts extracted from both control and fumigated samples varied with time of incubation and with sample type: the values in Table 3 vary appreciably. Levels of NO_3^--N (not shown) comprising about 2 per cent of the mineral-N in the peats and about 50 per cent in the mineral soil, showed little change following fumigation and subsequent incubation.

In contrast, large amounts of inorganic-P were extracted from all fumigated peats at day 0 enabling estimates of biomass-P to be calculated

Table 3. Biomass-C calculated from the Anderson and Domsch test, ATP content and the flush of CO_2-C and mineral-N. Biomass-N and -P calculated from F_n and F_p, respectively

Site	Date sampled	Biomass-C, g kg^{-1} d.wt.				Biomass-N g kg^{-1}	Biomass-P g kg^{-1}
		Method					
		Anderson and Domsch[1]	CO_2-C Flush[2]	Mineral-N Flush[3]	ATP content[4]		
1	a 3/81	2.85	3.20	2.54	2.47	0.79	ND
	a 2/82	1.09	0.64	1.66	ND	0.52	0.25*
2	a 3/81	3.59	2.78	5.42	0.79	1.69	ND
	b 10/81	3.90	No flush	3.67	ND	1.15	0.38
	c 2/82	2.52	0.37	1.41	ND	0.44	0.30*
3	a 1/80	1.00	0.68	0.62	ND	ND	ND
	b 3/81	0.36	0.41	ND	0.24	ND	ND
	c 10/81	1.81	0.82	1.57	ND	0.49	0.13
4	10/80	0.50	No flush	1.90	ND	0.59	0.10
5	10/80	2.76	2.64	3.28	ND	1.03	0.11
6	11/80	0.44	0.44	0.84	0.23	0.26	ND

ND Not determined
* Corrected for P-recovery
Assumptions
[1] 1 ml $CO_2 \equiv$ 40 mg biomass-C
[2] $k_c = 0.45$
[3] Biomass-C = 8 × F_n
[4] Biomass-C = ATP × 138

(Table 3). Amounts of inorganic-P extracted in 0.01 *M* $CaCl_2$ from fumigated larch humus (Site 2) varied appreciably between samples taken at different times. In subsequent tests with added K_2HPO_4, 0.01 *M* $CaCl_2$ extracted 96 and 56 per cent of inorganic-P added to pine (Site 1b) and larch humus (Site 2c) respectively. Allowance for poor recovery in the latter improved estimates of biomass-P in larch humus (Table 3). For the mineral soil (Site 6), inorganic-P levels in 0.01 *M* $CaCl_2$ extracts of both untreated and fumigated samples were below the detectable limit (< 0.1 mg kg^{-1}) and recovery of added phosphate was less than 10 per cent. Therefore, in this soil it was not possible to estimate biomass-P using this extractant (Table 3).

Most of the oxidizable-C, soluble- and mineral-N and inorganic-P released by fumigation of pine and larch humus was formed after only 2 hr of treatment (Figs. 3 and 4), and a noticeable effect on inorganic-P was detected after only 15 min. For Scots pine humus (Site 1b), subsequent incubation at 25°C for 10 days enhanced the release of CO_2-C and inorganic-P relative to the unfumigated control but in larch humus (Site 2c), over the same period, the flush of inorganic-P

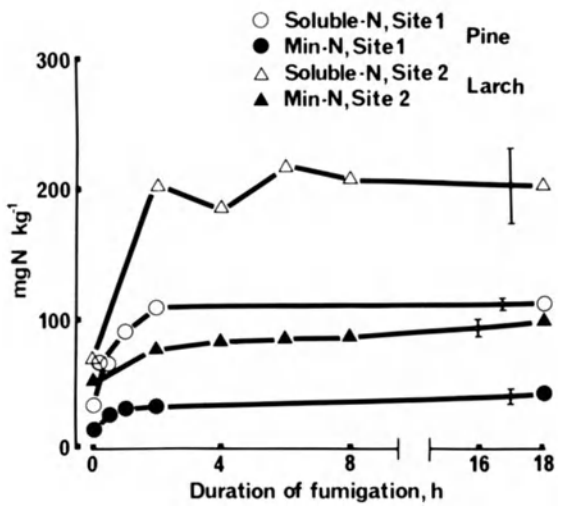

Fig. 3. Effects of duration of fumigation with $CHCl_3$ on the levels of soluble- and mineral-N extracted from samples of larch and pine humus immediately after treatment. Bars indicate SD on a mean of 3 replicate samples.

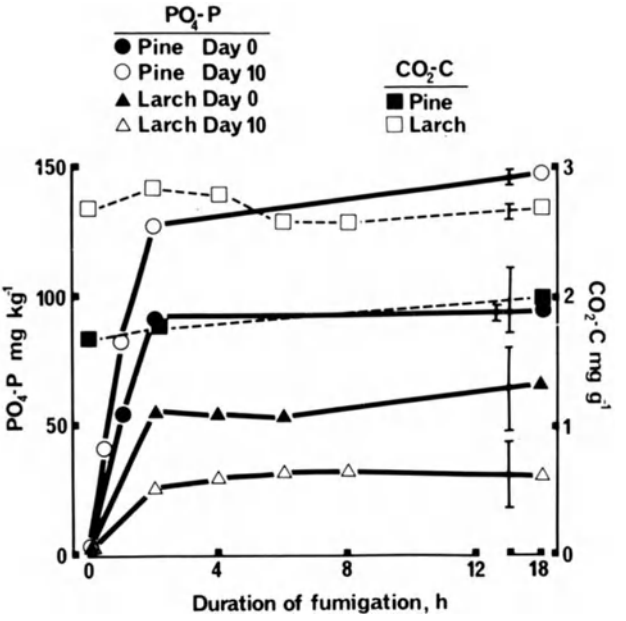

Fig. 4. Effects of duration of fumigation with $CHCl_3$ on the levels of inorganic-P released from samples of pine and larch humus immediately after treatment and after 10 days incubation at 25°C. CO_2-C released measured at intervals during incubation. Bars indicate SD on a mean of 3 replicate samples.

diminished slightly. Fumigation had a greater effect on soluble- than on mineral-N indicating that the treatment markedly increased the amounts of organic-N extracted by 1 *M* KCl (Fig. 3). Incubation increased soluble-N levels in both control and fumigated samples without significantly changing F_n (not shown).

Values for biomass-C, calculated from F_c and F_n for the various experiments, were compared with estimates obtained by the Anderson and Domsch test and with ATP contents (Table 3). Determination of ATP contents had a high variability (average CV 19 per cent) and did not relate to levels of biomass-C obtained by the Anderson and Domsch test.

Discussion

Peats and mor humus all showed increases in the extractable amounts of mineral-N and inorganic-P immediately after fumigation yet estimates of biomass-C based on the flushes of CO_2-C and mineral-N during subsequent incubation did not agree with values obtained by the Anderson and Domsch test (Table 3). The accuracy of the Anderson and Domsch test when checked against estimates of fungal and bacterial biovolumes in 3 organic soils was reasonable provided that only those organisms with viable cell contents were considered to contribute to the active fraction (Sparling, Williams, unpublished). Thus the anomalous results are probably a reflection of the influence of the high C:N ratios in these soils on CO_2-C and mineral-N release from untreated samples during incubation. Indeed, Jenkinson and Ladd[12] do not recommend the fumigation method for soils rich in readily decomposable material because of the effects of decomposition and N immobilization in the untreated samples on the measurement of F_c and F_n. Furthermore, acidity may limit the rate of decomposition of killed microbial cells in these soils[11] but attempts to enhance the flush of CO_2-C by additions of lime, nutrients and mineral soil were confounded by changes in both control and fumigated samples leading to erratic results (Sparling, Williams, unpublished).

Fumigation consistently increased the amounts of inorganic-P extractable immediately after treatment and for the three humus samples calculated values of biomass-P were related in general with biomass-C measured by the Anderson and Domsch test. Biomass-P values in the 3 peat soils were lower than in mor humus and remarkably similar despite differences in biomass-C values and total N and P contents. The microbial origin of the inorganic-P flush was confirmed by the similarity in the amounts of inorganic-P released from γ-irradiated

(2.5 Mrad) and from irradiated and fumigated pine humus, *i.e.* 86 ± 10 and 96 ± 10 mg P kg^{-1} dry wt., respectively. All biomass-P values in peats and humus (Table 3) are greater than those obtained for mineral soils[5,9]. Using biomass-C values obtained from the Anderson and Domsch test, the calculated C:P ratios for biomass, ranging from 4.5 to 25.6, are considerably lower than the value of 50 suggested by Jenkinson and Ladd[12], but are closer to the range (6.7 to 12.5) obtained for fungal material[2]. The calculated amounts of biomass-P (Table 3) comprise a remarkably high proportion of the total soil-P, ranging from 7–22 per cent, compared with the much lower values in mineral soils[5]. However, this proportion in peat and humus is similar to the biomass-P in mineral soils expressed as a percentage of organic-P, especially in grassland with a high organic matter content.

Our conclusions, from this limited number of samples, are that fumigation has a marked effect on extractable-N and -P and, at present, inorganic-P probably offers potentially a better basis for estimates of microbial biomass in acid organic soils than either C or mineral-N flushes since measurements can be made immediately after treatment. Further studies on the relationship between inorganic-P flush and the microbial biomass are reported elsewhere (Sparling and Williams, in preparation). The soluble-N flush immediately after fumigation may be another measurement on which to base calculations of microbial biomass, provided that the appropriate conversion factors can be derived to relate extractable soluble-N levels to the original biomass.

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Mineralization dynamics in fallow dryland wheat plots, Colorado

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Key words Inorganic nutrients Microbial biomass Mineralization Soil fauna Tillage practice

Summary There was a flush of mineralization in fallow wheat plots in the wet and warm summer of 1982 at Akron, Colorado. Peak mineralization rates and concentrations of N and P coincided with a 2.5-fold increase in protozoan biomass. No-till contained considerably more activity than stubble mulch plots, especially in the surface 2.5 cm and there was more water storage in no-till on all dates. Differential management of agricultural residues and the resultant effects upon the microbial community significantly altered patterns of nutrient cycling.

Introduction

The purpose of fallowing in dryland wheat agriculture is to accumulate sufficient soil moisture over a two-year period to produce an acceptable yield in regions where there is usually not enough rain in one year to support a crop. Fallowing has many secondary effects, some of which need consideration when evaluating the overall efficacy of this practice. For example, increased loss of soil organic matter caused by more favorable moisture and temperature for microbial activity will result in increased mineralization and subsequent loss of inorganic forms of macronutrients under fallow conditions. Fallowing may also accelerate nutrient loss via wind and water erosion. New management practices are being developed to reduce these losses.

The fallow rotation is managed to control weeds so stored water is not transpired before the crop can utilize it. *Stubble mulch* refers to weed removal by the use of a mechanical device (not plowing; *e.g.*, rod weeder, sweep or tandem discs) that incorporates a portion of the crop residues into the soil with each tillage. *No-till* refers to control of weeds by herbicides where the only disturbance of the soil surface occurs during planting. Each of these practices disturbs the soil's physical, chemical, and biological properties.

Under no-till management soil surfaces are cooler and wetter than under stubble mulch because of greater solar reflectance. Residues are not mixed as intimately with the soil under no-till. These effects

delay mineralization in the spring but prolong it through the summer of fall. Delayed mineralization during the fallow period of the no-till makes nutrients available nearer the time of plant uptake after fall planting, so decreasing the probability of leaching losses thus maintaining a less leaky nutrient cycle. It is the response of the biological community to these differences in tillage which results in differential mineralization. We studied the microbial community, including soil fauna, as well as soil nutrient contents, sampling at frequent intervals (3–4 weeks) to determine the dynamics of each group relative to the type of tillage practice used. Fallow agroecosystems provide an excellent milieu to study the biological mediation of mineralization, because the absence of plant growth allows measurable accumulation of mineral nutrients.

This short paper summarizes the trends observed during a summer flush of biological activity in the fallow phase, the data being a subset of a larger experiment lasting from harvest to harvest (~ 14 dates) over both the fallow and cropped phases of the rotation.

Materials and methods

Samples were taken from long-term tillage experiment plots (16 years) at the Central Great Plains Research Station, located at Akron, Colorado, USA. Six 5 cm diameter cores were bulked from each of the three field replicates for the fallow plots of stubble mulch and no-till treatments. Increments of 0–2.5, 2.5–5.0, and 5–10 cm were sampled at five dates between 8 June and 13 September 1982.

Acari and Collembolla were extracted using high-gradient Tullgren extraction funnels[6]. Nematodes were extracted using Baermann funnels after wet sieving[3]; only non-root feeding forms are discussed here. Protozoa were estimated according to Darbyshire *et al.*[2]. Microbial biomass C and N were estimated using the chloroform method[5], as modified by Voroney and Paul[8]; field samples were brought to field capacity before incubations. Microbial P was measured by the method of Hedley and Stewart[4].

Respired C was measured in the unchloroformed controls of the microbial C method for 0- to 10- and 10- to 20-d periods. Mineralizable N was the amount of NO_2 ions that accumulated between days 0 and 20 for the unchloroformed control of the microbial N method. Inorganic and organic P were determined by extraction with $NaHCO_3$, pH 8.5[7]. NO_3 and NH_4-N concentrations were determined on 2 *M* KCl extracts.

Concentration values ($\mu g\, g^{-1}$ soil) were converted to an areal basis ($kg\, ha^{-1}$) using bulk density information and summing over the top three depths to obtain $kg\, ha^{-1}$ to 10 cm.

Results

The mean air temperature (7 d running average, °C), rainfall events ($mm\, d^{-1}$), and millimeters of available water in the top cm (exclusive of > 1500 k Pa suction water) for the no-till and stubble mulch treatments at each of the 5 sample dates are portrayed in Fig. 1. The no-till treatment always contained more water in the top 10 cm than the stubble mulch treatment, especially on 6 July when there was the least

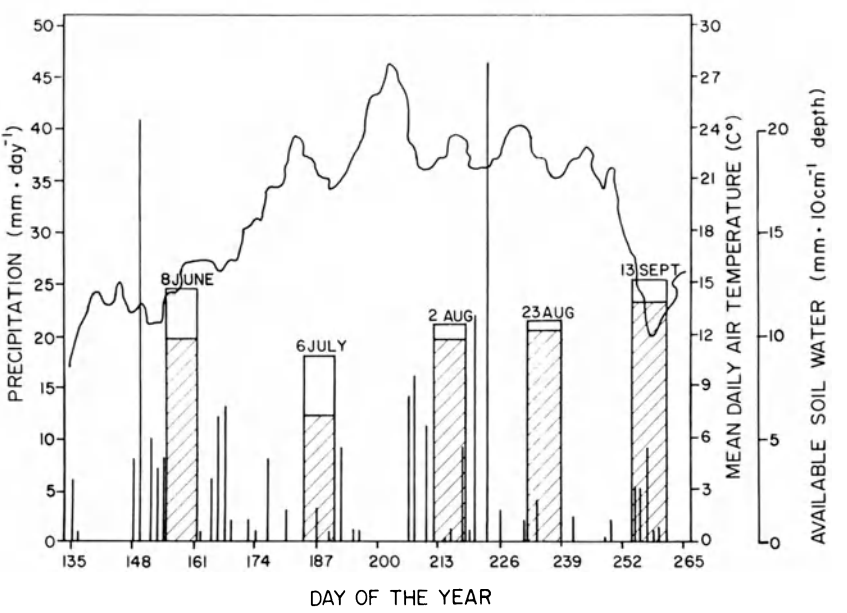

Fig. 1. The climatic regime 15 May–22 September 1982. Sample dates are designated by the histograms, which record millimeters of available ($\leqq$ 1500 k Pa suction) water in the top 10 cm of soil in stubble mulch (hatched) and the extra in no-till (open) treatments. Vertical lines are daily rainfall events (mm d^{-1}), and the solid line represents the daily mean air temperature (7 d running average).

amount of water present (Fig. 1). The year of this study was an unusually wet but warm year, but occasionally the surface layers became quite dry.

Seasonal soil faunal C in the top 10 cm (kg C ha^{-1}) varied differently for soil microarthropods, holophagous nematodes, and protozoa. Microarthropod biomass was greatest on the first two dates; holophagous nematodes on the first and third dates for stubble mulch and second date for no-till; and protozoa on the first, fourth, and fifth dates (Table 1a). Biomass C patterns between the stubble much treatments differed for microarthropods and nematodes, with the stubble mulch treatment having two peaks of biomass (dates one and three), while the no-till had its highest values on the second date for nematodes and first and second dates for Acari.

Seasonal microbial C and P dynamics were more closely coupled than microbial C and N (Table 1b). There was a large decline in microbial C and P, followed by an increase on 2 August. Microbial C was higher in the no-till than in stubble mulch for the first three dates while microbial N generally decreased from spring through fall. Microbial P did not differ between tillages.

Respired C, in the unchloroformed sample was greater for the first

Table 1. Faunal C, Microbial biomass, Mineralized C and N, N and P under stubble mulch and no-till treatments of the fallow phase of dryland wheat plots.
a) Soil fauna biomass C (kg C ha^{-1} to 10 cm) for four categories. (Note differences in the multiplier for each category).
b) Microbial biomass C, N, and P (kg element ha^{-1} to 10 cm)
c) Mineralizable C (CO_2-C) and NO_3-N as kg ha^{-1} to 10 cm in unchloroformed 0–10 or 10–20 d incubations of soils sampled from the field.
d) NH_4-N, NO_3-N, and extractable inorganic and organic P amounts (kg ha^{-1}) in the top 10 cm of soil.

Variable	Treatment	Date					ANOVA Effect	Probability
		8 June	6 Jul	2 Aug	23 Aug	13 Sep		
a) Faunal carbon								
Collembola	Stubble mulch	6.69	1.03	2.18	0.61	1.49	T	.002
							D	.001
X 100	No till	5.58	8.29	4.01	1.60	1.49	T X D	.019
Acari	Stubble mulch	3.77	0.47	0.91	0.40	0.92	T	.014
							D	.003
X 10	No till	3.60	3.31	1.24	1.32	0.54	T X D	.020
Holophageous nematodes	Stubble mulch	0.88	0.46	1.12	0.51	0.56	T	.418
							D	.006
X 10	No till	0.48	1.69	0.96	0.57	0.32	T X D	.002
Protozoa	Stubble mulch	1.76	0.67	0.74	1.96	1.92	T	.278
							D	.001
X 1	No till	2.20	0.96	0.93	2.29	1.61	T X D	.669
b) Microbial biomass								
Carbon	Stubble mulch	245	204	271	186	255	T	.054
							D	.019
	No till	329	273	299	194	256	T X D	.532
Nitrogen	Stubble mulch	81	57	53	60	56	T	.289
							D	.001
	No till	92	72	49	62	48	T X D	.115
Phosphorus	Stubble mulch	5.7	7.5	10.1	4.6	7.2	T	.902
							D	.196
	No till	5.5	6.8	10.1	4.9	7.1	T X D	.999
c) Mineralized C and N								
Respired C	Stubble mulch	52	77	57	42	68	T	.042
							D	.075
(0–10 d)	No till	97	110	98	41	67	T X D	.495
Respired C	Stubble mulch	49	42	56	53	24	T	.019
							D	.023
(10–20 d)	No till	80	69	84	57	24	T X D	.525
Mineralizable N	Stubble mulch	10.63	6.39	5.79	2.62	1.95	T	.022
							D	.130
(0–20 d)	No till	12.68	5.79	8.90	−3.3	−1.38	T X D	.443
d) N and P								
NH_4^+-N	Stubble mulch	3.1	1.0	2.0	1.8	0.8	T	.012
							D	.001
	No till	4.0	1.8	4.6	1.2	1.4	T X D	.050
NO_3^--N	Stubble mulch	7.2	10.6	16.3	17.8	16.1	T	.001
							D	.001
	No till	7.8	23.8	21.6	45.1	46.2	T X D	.016

Table 1. Continued

Variable	Treatment	Date					ANOVA	
		8 June	6 Jul	2 Aug	23 Aug	13 Sep	Effect	Probability
$PO_4^{\equiv}$-P	Stubble mulch	18.1	17.6	22.2	19.8	18.7	T	.001
							D	.060
	No till	21.0	21.9	24.7	26.4	22.9	T × D	.511
Extractable organic-P	Stubble mulch	–†	3.69	5.90	7.00	6.89	T	.954
							D	.001
	No till	–	2.45	6.69	7.93	6.89	T × D	.440

† Not measured on this date.

incubation period than for the second and no-till was greater than stubble mulch for the first few dates (Table 1c). Mineralizable N decreased with successive sample times, and some negative values were obtained on the last two dates of the no-till sampling.

Amounts of NH_4^--N were greatest on dates one and three, with more in no-till than in stubble mulch for the first three dates (Table 1d). Nitrate levels increased more rapidly under no-till. Inorganic P levels peaked on dates three and four in both tillages but there was always more in no-till. Extractable organic P did not differ between treatments but demonstrated a marked peak on the fourth date, with some decline in the no-till by the fifth date.

Discussion

The cool spring followed by the warm, wet summer created a synchronized flush of biological activity in August. On the driest date (6 July) there was less activity because of lack of water, especially in the stubble mulch. There was more available water in the no-till than in stubble mulch treatment (17% greater averaged over all dates). On 6 July there was 42% more available water; the surface layer of the stubble mulch had no available water (> 1500 k Pa suction). Soil fauna responded to this difference in water content: collembola, mites, and nematodes in the stubble mulch treatment showed a decrease on 6 July, followed by an increase on 2 August but no-till showed no such decline (Table 1a). There was also a depression in microbial biomass C on 6 July for both tillages. However, because of the unusually high rainfall, there were higher than average amounts of water present, which facilitated biological development throughout the summer.

There was a temporal displacement of soil fauna groups (Table 1a). Microarthropod biomass was highest early, nematodes at intermediate

times, and protozoa at early and late dates. This sequence may relate to the trend of microfloral increase during utilization of the straw residues, and subsequent secondary decomposition of microbial biomass resulting in mineralization. Soil protozoan biomass C was seven times greater than that of all other faunal groups combined (Table 1a). Only total protozoa were measured, so it is not known how many were cystic, but the increase from 2 to 23 August was that of a rapidly expanding population, probably with few cysts present.

Perhaps the most intriguing result of our sampling is the lack of correlation between microbial biomass C and N on 2 and 23 August. Both biomass C and P showed large declines on 23 August, but biomass N showed little change (Table 1b). The mean biomass C:N ratio for all dates and treatments was 4.0, and the C:P ratio was 36:1. Between 2 and 23 August the C:P changed from 28.2 to 35.7, a 26% increase; but the C:N changed from 5.6 to 3.1, a decrease of 45%, mostly because of decreases in microbial C. The microbial C estimates could be too high because of problems in analytical separation of substrate C and microbial C in sieved and moistened soils (Voroney and Paul, in press). However, this change in C:N ratio may indicate a shift from a fungal to a bacterial dominated microflora. The largest increase in soil faunal biomass also occurred between 2 and 23 August, with protozoan biomass C increasing 2.5-fold (Table 1a). The highest N mineralization rates and highest extractable inorganic and organic P concentrations also occurred in the field at this time. NH_4-N concentrations were highest on 8 June, when cool temperatures may have slowed nitrification, and on 2 August, immediately before the period of greatest NO_3 accumulation in no-till plots. Mineralizable N was negatively correlated with field NO_3-N over all five dates and both treatments ($r = -0.73$, Pearson r, $P \leqq 0.001$, $n = 30$).

The flush of mineralization occurred during a period of above average soil moisture in the 1982 season although there was more water stored under no-till than stubble mulch management. Large declines in microbial C and P, increases in mineral N and extractable inorganic and organic P concurrent with the increase in soil protozoa, suggest that protozoan activity may be an important component of mineralization under certain field conditions, as has been observed in laboratory experiments[1]. However, during the August flush of activity microbial N increased slightly concomitant with a decrease in microbial C resulting in a decreased C:N. Thus, at this time, microbial N must be cycling rapidly with high throughput but little net change or there is a flow of N to some unmeasured pool (*i.e.*, soluble N) immediately before mineralization.

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Dynamics of soil microbial biomass N under zero and shallow tillage for spring wheat, using ^{15}N urea

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Key words Biomass Chernozemic soils Chloroform fumigation Microbial biomass N ^{15}N-urea Spring wheat Urea Zero and shallow tillage

Summary Field studies to determine the effect of zero and shallow (10 cm) cultivation on microbial biomass were conducted on several Chernozemic soils in western Canada. Using the $CHCl_3$ fumigation method, the distribution of microbial biomass N and the immobilization and subsequent release of added ^{15}N (^{15}N-urea) from the microbial biomass were determined in the A horizon, at the 0 to 5 and 5 to 10 cm depth, during the growing season for spring wheat.

Temporal variation in microbial biomass N, associated with the development of the rhizosphere, was characterized by an increase between Feekes stage 1 and 5 or 10 and decrease at Feekes stage 11.4. Over the long term, the variation in biomass N between tillage systems corresponded with crop residue distribution. Immobilization of fertilizer N was related to the increase in biomass N from Feekes stage 1, which in turn, was associated with the incorporation of recent crop residues or levels of labile organic matter in the surface soil. The study demonstrated the relatively rapid remineralization of immobilized fertilizer N under field conditions and emphasized the role of the microbial biomass N as both a sink and source of mineral N.

Introduction

The redistribution of biological properties and activity with depth in the soil under zero tillage, in comparison to moldboard ploughed systems has been of current interest[2,9]. Recently, Carter and Rennie[1] compared zero and shallow tillage, the latter being commonly used for spring wheat production on the Canadian prairies, and showed that marked differences in the distribution of microbial biomass had occurred in the 0 to 10 cm depth.

In addition to cultivation, the development of the rhizosphere during crop growth has been associated with temporal variations in microbial biomass[7]. Furthermore, the perturbations of the surface soil micro-environment may also influence biomass size. In this study, the combined effect of crop growth and cultivation on the microbial biomass was evaluated at specific growth stages of spring wheat under zero and shallow tillage systems. The immobilization and release of both soil and fertilizer N by the microbial biomass was determined to assess the turnover of N in the biomass over time.

Materials and methods

Tillage sites and microplots

The study was conducted in 1981 and imposed upon existing zero and adjacent shallow (5 to 10 cm, using a cultivator or disc) tillage systems which had been established on Chernozemic soils in western Canada for various lengths of time. The tillage comparisons were located at Scott (Scott loam, 2 years), Watrous (Elstow clay loam, 4 years), Melfort clay, 4 and 12 years), Saskatchewan; and Lethbridge (Lethbridge loam, 16 years) Alberta. The 2 to 4 year tillage comparisons were cropped to continuous spring wheat, while the 12 and 16 year studies were a spring wheat-fallow cropping system. During the fallow year only herbicides (chemical fallow) were used on the zero tillage, while the conventional system received several shallow tillage treatments. Certain soil properties for the 0 to 10 cm depth are given in Table 1. Agronomic aspects have been discussed previously[1]. In this study, soil moisture content was generally similar between tillage systems; while mean daily maximum soil temperatures (for the seed row at 5 cm depth) were 1 to 5°C higher for the shallow compared to the zero tillage at Feekes stage 1 and 5 (unpublished data). Growing season precipitation ranged from 156 mm at the Melfort sites (below normal in May and June) to 297 mm at Lethbridge.

Ten galvanized open steel cylinders (30 cm internal diameter, 15 cm high) were pushed 12 cm into the soil on a 4 m grid, on each tillage system at Feekes stage 1. The use of wide shallow cylinders enabled a relatively large area to be sampled without causing significant soil disturbance or compaction. To introduce labelled N into the microbial biomass, urea with a percent ^{15}N excess of 5.526 was broadcast in solution form, within the cylinders, at a rate equivalent to 100 kg N/ha. After application, 1 cm of H_2O was added to each cylinder to ensure movement of the added N off the residue layer into the soil. Two cylinders were removed just prior to the ^{15}N application at Feekes stage 1 and further pairs, after the N application, at Feekes stage 5 and 10. The remaining 4 cylinders were removed at harvest (Feekes stage 11.4).

Microbial biomass determination

Cylinders removed at the specific crop stages were taken to the laboratory and the soil volume separated into the 0 to 5 and 5 to 10 cm depth. After removal of crop residue, the soil was passed through a 8 mm sieve and two 50 g (ODW basis) soil samples from each depth were immediately fumigated with $CHCl_3$ (Jenkinson and Powlson[3]) without adjusting the H_2O content of the soil. After fumigation, the soil samples (plus an unfumigated soil sample) were adjusted to 55% of their H_2O holding capacity and incubated at 25°C for 10 days. After incubation the accumulated NH_4-N was extracted with 2*M* KCl and distilled in duplicate 50 ml aliquots using MgO. The distillate was concentrated by evaporation under vacuum prior to isotope analysis on a Micromass 602E automatic mass spectrometer. Only the Melfort soils (at Feekes stage 5 and 10) showed any NH_4-N in the unfumigated soil. Thus, for the majority of samples, the accumulated NH_4-N would correspond to the flush (fumigated minus unfumigated) of mineral NH_4-N. Since values for k_N are variable, emphasis was placed on the flush of mineral NH_4-N as a measure of microbial N content between soils.

Previous studies[1] on these soils (0 to 10 cm) obtained a ratio of 4.4 (0.2 SE) between the flush of CO_2-C and NH_4-N after fumigation. Thus, multiplying the NH_4-N flush by 9.8 (*i.e.*, 4.4/0.45, 0.45 being the k_c factor for 25°C) provided an indirect approximation of biomass C.

Results

Effect of crop growth on microbial biomass

Significant temporal variations in the biomass N and calculated C content were observed over the growing season (Fig. 1). At both soil depths they increased from Feekes stage 1 to a maximum generally between Feekes stage 5 and 10; followed by a decline at Feekes stage

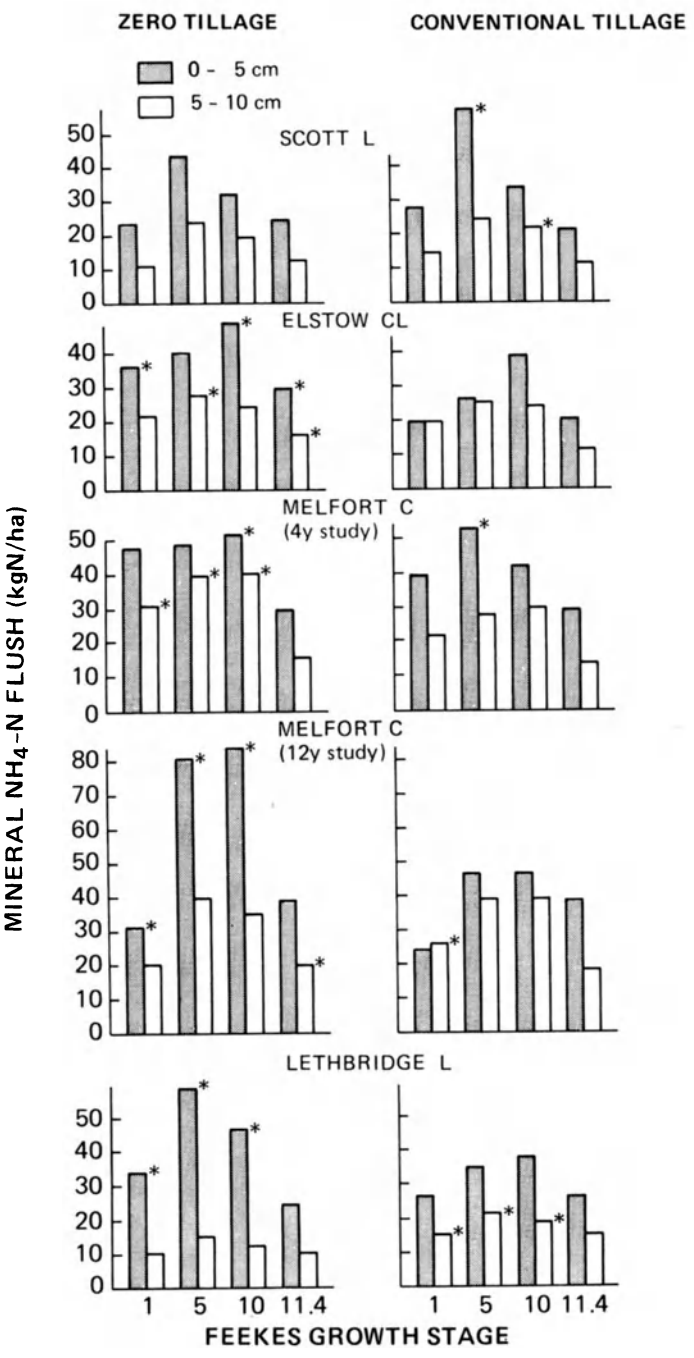

Fig. 1. Change in the mineral NH_4-N flush over the growing season, at two soil depths, under zero and conventional shallow tillage (* indicates significant difference between tillage systems, $P = 0.05$).

11.4. The percent soil organic N in the mineral NH_4-N flush ranged from 0.9 to 3.2 at Feekes stage 1 and increased from 1.5 to 5.6 at Feekes stage 5.

Effect of cultivation on microbial biomass

At the Feekes 1 growth stage, before substantial plant growth had occurred differences in the distribution of biomass N with soil depth were evident between tillage systems. These differences were maintained for most of the growing season at the 0 to 5 cm depth at the Elstow and Melfort soil sites, and for both soil depths on the long-term Lethbridge soil site (Fig. 1).

Immobilization of the added fertilizer N into the microbial biomass occurred mainly in the 0 to 5 cm depth and generally showed a maximum at Feekes stage 5, then declined (Fig. 2). However, at the Melfort sites low crop utilization and impeded distribution of the fertilizer N, due to low rainfall during the early growing season, delayed maximum immobilization of ^{15}N until the initiation of adequate rainfall.

The increase in the mineral NH_4-flush from Feekes stage 1 to 5 (Fig. 1) and subsequent recovery of fertilizer N in the biomass (Fig. 2), for the 0 to 5 cm soil depth, was greater under shallow compared to zero tillage for the continuous wheat studies (Scott-2 y and Elstow-4 year). In contrast, the older wheat fallow systems (Melfort-12 y and Lethbridge-16 year) showed the reverse situation.

Microbial biomass turnover

The decline in the mineral NH_4-flush after Feekes stage 5 or 10 allowed the estimation of approximate half-life values over all sites, (excluding Melfort-4 year) for the total NH_4-N flush, the increased N content associated with rhizosphere development after Feekes stage 1 and the fertilizer N content of the mineral NH_4-N flush (Table 2). These values varied across the different locations and did not show consistent differences between tillage systems. The overall means for locations and both tillage systems gave half-lives of about 60 days (34 to 124 range for the 0 to 5 cm depth) for the total mineral NH_4-N flush, and 20 days (4 to 59 range) for both the mineral NH_4-N flush increase and the fertilizer N content.

Discussion

The changes in microbial biomass over the growing season agree with other studies in England[7], being related to the rhizosphere and root exudates with modification due to soil moisture and temperature.

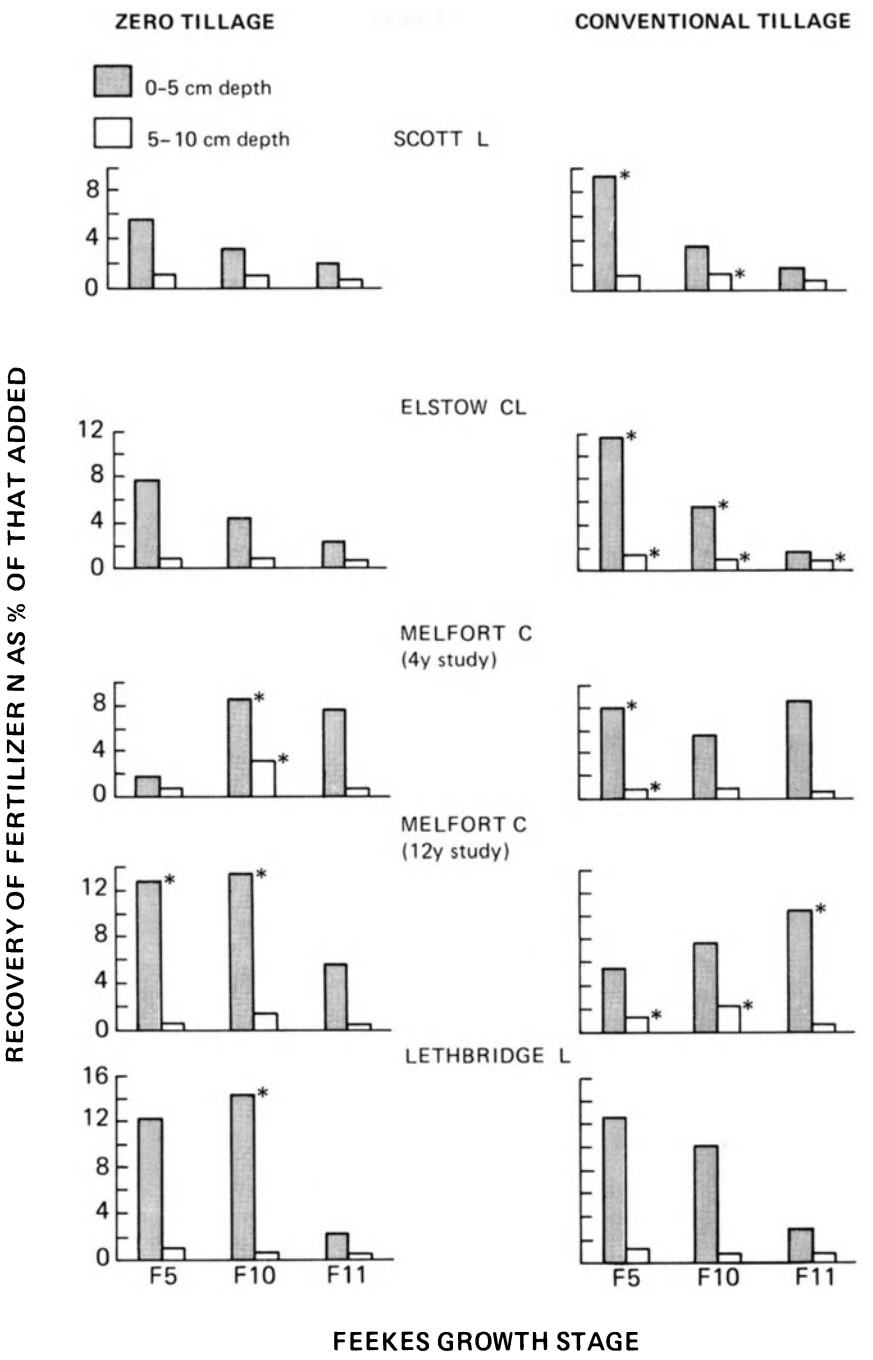

Fig. 2. Recovery of fertilizer N, as a percentage of that added, in the mineral NH_4-N flush over the growing season, at two soil depths, under zero and conventional shallow tillage (* indicates significant differences between tillage systems, $P = 0.05$).

Table 1. Soil properties* for the 0 to 5 and 5 to 10 cm depth at each tillage site**

	Scott		Elstow		Melfort-4		Melfort-12		Lethbridge	
	0–5	5–10	0–5	5–10	0–5	5–10	0–5	5–10	0–5	5–10
Bulk density (MgM^{-3})	1.24	1.13	1.05	1.05	0.99	1.03	1.06	0.89	1.34	1.20
pH H_2O (1:2.5)	5.9	5.8	6.4	6.2	5.8	6.0	6.2	6.0	7.2	7.2
EC (dSm^{-1})	0.10	0.13	0.20	0.16	0.45	0.16	0.38	0.21	0.15	0.16
Total N (%)	0.263	0.250	0.261	0.249	0.469	0.437	0.487	0.504	0.159	0.155
Organic C (%)	2.601	2.324	2.538	2.392	4.596	4.309	4.865	4.717	1.565	1.457
Clay, < 2 um (%)	19.4	19.6	31.6	32.6	51.3	51.9	58.9	57.9	27.7	28.6

* Since values were similar between tillage systems the means were averaged at each site

** All soils were classified (USA) as Typic Boroll, except for the Udic boroll soil at Melfort

Table 2. Average half-life* for the net decline of total N content, increased N content due to cropping, and fertilizer N content of the mineral NH_4-N flush

Depth (cm)	Total N content	Increased N content**	Fertilizer N content
		half-life (days)	
0–5	54 ± 7	20 ± 5	19 ± 3
5–10	68 ± 6	20 ± 7	ND

* Mean and SE (for both tillage systems and all sites except Melfort-4). Calculated from date of Fig. 1 and 2.
** Increase over Feekes stage 1.
ND, not determined, too low.

The differences in microbial biomass with depth prior to plant growth, reflect the distribution of crop residues and labile organic matter in the surface soil due to tillage differences as discussed by Carter and Rennie[1]. For the long term tillage comparison at Lethbridge, the differential influence of both the rhizosphere and crop residues distribution on the biomass was observed over the entire growing season. In contrast, for the Scott site the lack of conclusive biomass response to tillage differences is probably related to the short duration of the tillage comparisons.

The differing increases of the microbial biomass N and ^{15}N immobilization between tillage systems, at the early part of the growing season (from Feekes stage 1 to 5) is probably related to the recent incorporation of crop residues. For the continuous wheat systems, the conventional shallow cultivation allows greater residue incorporation than the zero tillage system. In contrast, for wheat-fallow systems, the preceding fallow year can modify crop residue levels. Recent studies (Lindwall and Anderson[6]) indicated that surface residues can be reduced for the conventional and chemical fallow, by 87% and 30% respectively. Thus, the greater residue levels following the chemical fallow would probably account for the greater increase in microbial biomass N and immobilization of ^{15}N (at the 0 to 5 cm depth) under zero tillage compared to the conventional tillage.

Widely fluctuating moisture and temperature at the soil surface would induce a variable half-life for the microbial biomass. Under field conditions a propensity for relatively rapid mineralization of formally immobilized fertilizer N would exist. Thus the role of the microbial biomass can change from a sink to a source of labile N. Ladd *et al.*[5] obtained a half-life of 100 days for the initial net decomposition period (180 days) of the microbial biomass N: the overall half-life, however, measured over a four year period approximated 4 years. In this study, between 2 and 10% of the fertilizer N remained in the mineral NH_4-N flush (averaging 35% of residual fertilizer N) at the end

of the growing season. Subsequent studies showed that 20 to 40% of this residue fertilizer N in the biomass was released over a 100 day incubation period (unpublished results). Thus, the mean 60 day half-life appears to reflect the initial net decomposition phase of the total mineral NH_4-N flush.

The shorter 20 day half-life for both the NH_4-N increase and fertilizer N content may be related to the differential population response of various components of the biomass. Lynch and Panting[8] used the functional concepts of 'autochthonous' and 'zymogenous' to discuss both the relatively stable biomass and the increased biomass due to plant root exudation. The latter, being sensitive to levels of available energy, would probably have a shorter half-life than the stable biomass. Thus the increased biomass N associated with the growth of roots may be considered as a temporary sink for immobilized fertilizer N.

Acknowledgements The authors wish to acknowledge personnel and scientists of the Agriculture Canada, Research Stations at Melfort and Scott, Saskatchewan, and Lethbridge, Alberta; and the Saskatchewan Wheat Pool for permission and assistance to carrying out experiments on their tillage studies.

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Mineralization of C and N from microbial biomass in paddy soil

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Key words Biomass Carbon Chloroform fumigation Drying Mineralization flush Nitrogen Paddy soil

Summary Soil samples of paddy fields with different fertilizer managements in Yamaguchi Agricultural Experiment Station, Japan were used to investigate the contribution of microbial biomass to the pool of mobile plant nutrients in paddy soil. The quantities of nutrients mobilized in soils which had been fumigated or dried were closely related to the quantities available in freshly killed biomass. A "K_N-factor" (28 days) of 0.24 for the proportion of total N mineralized from dead biomass in paddy soils was obtained. It was observed that the C to N ratio mineralized from freshly killed biomass by chloroform fumigation of paddy soils was nearly 10 under aerobic conditions. For an approximate calculation of biomass C from the flush-N by chloroform fumigation of paddy soils, the equations of (B = 33 Fn, 10 days) and (B = 26 Fn, 28 days) were indicated. In oven-dried (70°C, 24 h) and rewetted soils, about 66% of N was mineralized from the freshly killed biomass during 28 days of incubation and the remaining 34% was derived from non-biomass organic matter of paddy soils.

Introduction

When soil is stressed, *i.e.* dried, frozen, fumigated or ground, a portion of the biomass is killed and the dead biomass is readily decomposed by surviving microorganisms[4,6,10,16]. Marumoto *et al.*[11,12] found that the quantities of nutrients mobilized in two German upland soils were closely related to the amounts available in freshly killed biomass; and they suggested a scheme for the transformation of dead microbial biomass C and N in arable soil during 4 weeks. However, little information is available on the quantity and characteristics of nutrients released from dead microbial biomass in paddy soil. The quantities and characteristics of carbon and nitrogen mineralized from freshly killed biomass in three paddy soils with different fertilizer managements for 7 years (1977 –1982) are shown in this paper.

Materials and methods

Soils

Samples were collected in early November, 1982 from the paddy field after harvesting of rice in Yamaguchi Agricultural Experiment Station. After sieving (< 2 mm), soil was kept at a moisture content of about 50% of max. WHC and stored in polyethylene bags for 10 days at room temperature. Soil properties and fertilizer managements (1977–1982) are shown in Table 1.

Soil treatments and incubations

Control Samples of moist soil equivalent to 20 g dry weight were incubated in 500 ml bottles with gas exchange plugs for CO_2-C determination at 25°C ± 0.5 and 50% of max. WHC for 56 days.

Fumigation Soil samples were fumigated with alcohol-free $CHCl_3$ (Jenkinson and Powlson[7]), then reinoculated with 1% fresh original soil and incubated by the same procedure as described for the control.

Drying Soil samples were dried at 70°C for 24 h, then rewetted to 50% of their max. WHC, reinoculated with 1% fresh original soil and incubated as for the control during 28 days.

Duplicate soil samples were used for each treatment and incubations were done with 4 replications.

Measurement of soil microbial biomass C and calculation of biomass N

The microbial biomass C of soil samples was measured by three methods: $CHCl_3$ fumigation[7], ATP[17] and direct count[9]. Using a C to N ratio of 7 to 1 for microbial biomass, its N content was calculated from the analytical data provided by Anderson and Domsch[1].

Determination of CO_2-C and soil mineral N

CO_2-C evolved from the soil during the incubation period was absorbed by soda asbestos (Merck Co.) in two coupling U tubes connected with an incubation bottle for 15 minutes. The absorbed CO_2-C was determined gravimetrically. Mineral N was extracted with a 10% w/v KCl solution and determined by steam distillation[3].

Results and discussion

Quantity of microbial biomass

Table 2 shows the estimates of soil microbial biomass by the three methods. Although the amounts of biomass C differed between methods, the relative proportion between soils *A*, *B* and *C* were almost the same. According to the $CHCl_3$ method, soil *A* (PK plot) and soil *B* (NPK plot), having the same organic C content, showed equal amounts of biomass C. The amount in soil *C* (NPK plus manure plot) with the higher organic C content was nearly double that in the other two. This may indicate that N application without carbonaceous materials such as plant residues or manure affects the quantity of soil microbial biomass very little.

Flush of C and N mineralization by $CHCl_3$ fumigation and drying pretreatments

Flush of C and N mineralization by fumigation occurred in all soil samples during the first week of incubation and their mineralization gradually increased until 28 days after the treatment (Fig. 1). the amounts of C and N mineralized were in the order $C \gg B > A$. Their mineralization flush was closely related to the initial quantities of

Table 1. Soil properties and fertilizer managements (1977–1982)

Soil*	Fertilizer managements**	Org. C %	T–N %	Texture	CEC	Exchangeable bases				pH (H_2O)
						Ca	Mg	K	Na	
						meq/100 g d.s.				
A	– PK	1.3	0.13	SL	8.4	4.7	1.6	0.2	0.1	6.7
B	NPK	1.4	0.14	SL	8.5	4.2	1.4	0.1	0.1	5.8
C	NPK manure	2.0	0.19	SL	8.5	4.4	1.2	0.6	0.1	5.7

* Rice (June–Oct.) and barley (Nov.–May) may have been cultivated annually since 1977.
** 100 kg each of N, P and K per ha was applied to the soil before rice transplanting and 15 tonne of rice and barley (June and Nove.), respectively. Total nutrient contents of N, P, K, Ca and Mg in manure (mean values for 5 years) were 2.0 ± 0.4%, 0.4 ± 0.2%, 3.4 ± 1.0%, 0.6 ± 0.2% and 0.3 ± 0.2% on dry matter basis, respectively.

Table 2. Estimates of soil microbial biomass by different methods

Soil	Org. C %	From chloroform fumigation[a]	From direct counts[b]	From ATP content[c]	Soil org. C in biomass by fumigation %
			mg C/100 g d.s.		
A	1.3	60.2 ± 1.1[d]	77.8 ± 4.4	73.3 ± 0.7	4.7
B	1.4	58.0 ± 0.4	80.4 ± 6.5	ND[e]	4.1
C	2.0	107.6 ± 10.4	148.2 ± 13.7	128.3 ± 2.9	5.4

a) Calculated from the flush of carbon using Kc = 0.45 and CO_2-C evolved by fumigated soil in 0–10 day period less CO_2-C evolved by untreated soil in 10–20 day period (Oades, Jenkinson 1979)[2].
b) Assuming that the biomass has a specific gravity of 1.1 and contains 25% dry matter, of which 47% is C (Jenkinson and Ladd[5]).
c) Using a biomass-C/ATP ratio of 171 (Tate and Jenkinson[17]).
d) ± Standard deviation. e) Not determined.

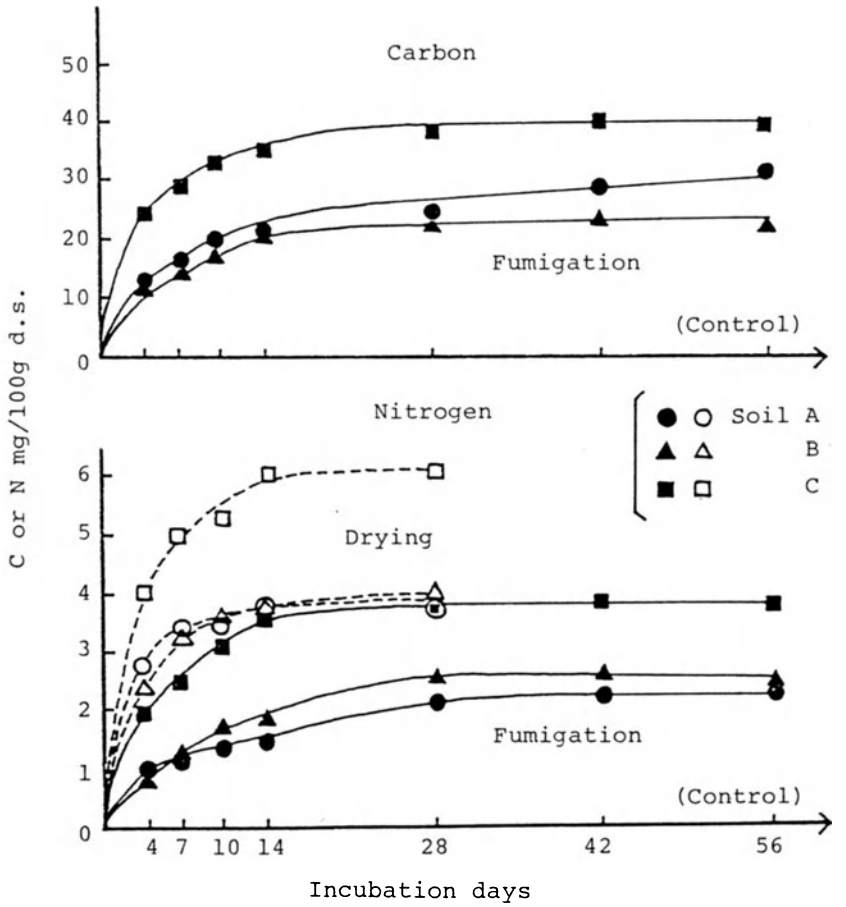

Fig. 1. Changes in C and N mineralized from dead microbial biomass by $CHCl_3$ fumigation during 56 days of incubation and N mineralization following drying (70°C, 24 h)-rewetting treatment during 28 days.

microbial biomass in the soil samples. The mean proportions of C and N mineralized from dead microbial biomass by fumigation for all soil samples were 38 ± 4% and 24 ± 2%, respectively. From the previous data[1,12], we suggested a "K_N-factor" (29 days) of 0.37 for the proportion of total N mineralized from dead biomass in arable soil but a "K_N-factor" of 0.24 for paddy soils was obtained in this experiment. The amounts of N mineralized from freshly killed biomass 28 days after fumigation were 2.3 mg 100 g^{-1} dry soil in soil *A*, 2.6 mg in *B* and 3.7 mg in *C*. The flush of N after drying (70°C, 24 h)-rewetting was greater than by fumigation for all soils. The amounts of organic N including microbial biomass N mobilized by oven-drying were 3.5 mg 100 g^{-1} dry soil in *A*, 3.7 mg in *B* and 6.0 mg in *C*.

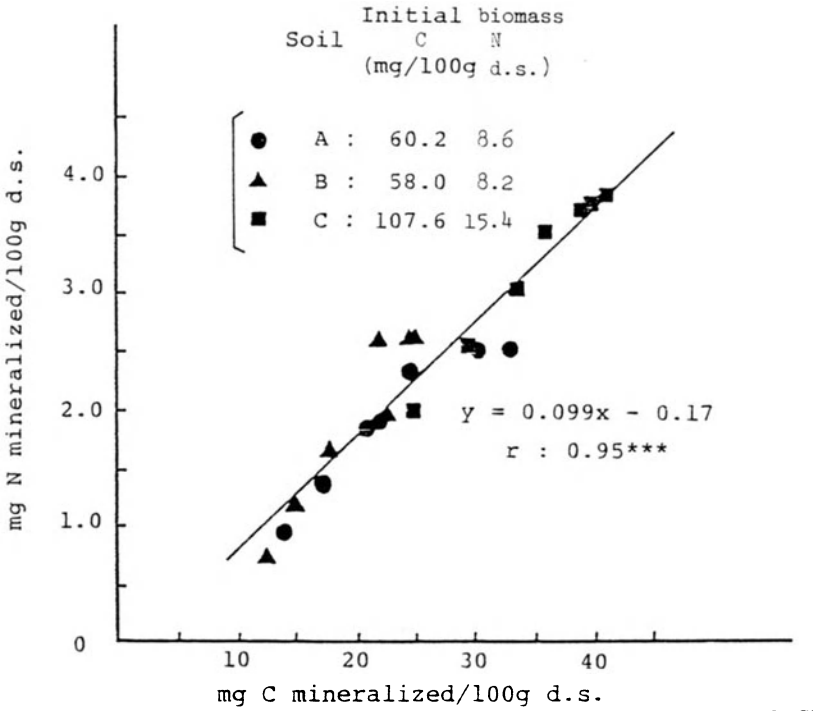

Fig. 2. Relationship between C and N mineralized from dead microbial biomass by $CHCl_3$ fumigation in paddy soils during 56 days of incubation.

Average yields of brown rice (tonne ha^{-1}/yr) during 1977 to 1982 was 3.5 ± 0.3 from soil *A*, 4.8 ± 0.3 from *B* and 5.2 ± 0.6 from *C*; and of barley 0.6 ± 0.2, 2.2 ± 0.7 ton and 2.3 ± 0.5, respectively. The crop yields depended partly on the amounts of N fertilizer applied to the soils, but also on the amounts of N mineralized from dead microbial biomass and from mobile soil organic matter during 28 days of incubation. This may indicate that the quantity of microbial biomass might be used as an index of soil fertility.

Relationship between C and N mineralized from dead microbial biomass in paddy soils

The ratio of C to N mineralized from dead microbial biomass by fumigation was nearly 10 in all three soils with differing quantities of initial microbial biomass (Fig. 2). Marumoto *et al.*[11] reported that the ^{14}C to ^{15}N ratio mineralized from dead labelled microorganisms in arable soil during 56 days was about 12 and also that the ratio of the decrease of biomass C to the amount of N mineralized after fumigation was about 10 in the associated work[12]. From these results, it is assumed that the C to N ratio mineralized from freshly killed soil

microbial biomass by $CHCl_3$ fumigation is nearly 10 under aerobic conditions. However, Powlson and Jenkinson[14] reported that the C to N ratio mineralized after $CHCl_3$ fumigation in six soils for 10 days ranged from 5.8 to 7.3. The difference between these sets of data needs consideration.

Relationship between the quantity of microbial biomass C and the flush of N mineralized following $CHCl_3$ fumigation of soil

Ayanaba *et al.*[2] suggested that a rough measure of biomass C (B) for tropical forest soils in Nigeria can be obtained by multiplying the N-flush (Fn) (after 10 days incubation at 25°C) by 8, using a K-factor of 0.5[7]. Marumoto *et al.* (1982b) also attempted an approximate calculation of biomass C from the flush-N after $CHCl_3$ fumigation of upland soils.

Table 3 summarizes the results obtained Ayanaba *et al.* and those for temperate soils in England[8] and in W. Germany[12] compared to current results for Japanese paddy soils. In both the German and Japanese soils the Fn factor was larger for 10 days than for 28 days. By calculating the biomass C using a K_C-factor of 0.45[13] and a ratio of 10 for the C and N mineralized from freshly killed biomass 10 days after fumigation, a theoretical equation of $B = 22$ Fn, can be obtained for arable soil. Further investigations are necessary to substantiate the accuracy of this equation.

Contribution of biomass N to plant nutrient pool in soil

Table 4 shows the percentages of the N-flush derived from dead microbial biomass and non-biomass by air and oven drying pretreatments of Germany and Japanese soils. Powlson[15] reported that about 36% of the flush-C of two English soils with *ca.* 25% of killed biomass was derived from dead biomass 10 days after grinding.

The W-Germany soils with *ca.* 39% of the biomass killed by air drying released about 55% of N-flush from dead biomass after incubation for 28 days, and about 76% of N-flush from *ca.* 64% of killed biomass after oven-drying[11,12]. Thus the proportions of N-flush derived from dead microbial biomass and from soil organic matter appear closely related to the percentage kill of the microbial biomass in soil.

The mean proportion of microbial biomass N in the total N-flush of the paddy soils was 66%: about 10% less than in the Germany soils after oven-drying pretreatment. The proportion may be affected by the incubation time, temperature, moisture content and the amount of organic matter in soil. However, these results demonstrated that the

Table 3. Relationship between soil microbial biomass C and the flush of mineral N following $CHCl_3$ fumigation

Soil and location	Incubation method	Incubation days	Measurement of biomass C	Relationship[a]
Tropical forest[b] soils (20 samples) in Nigeria	25°C ± 0.5 aerobic (55% WHC)	10	Fumigation[e] method	B = 8 Fn
Upland and grassland[c] soils (7 samples) in England	25°C ± 0.5 aerobic (50% WHC)	10	Fumigation[e] method	B = 7.7 Fn
Upland soils[d] (4 samples) in W-Germany	22°C ± 0.5 aerobic (34% WHC)	10	Respiration[f] method	B = 24 Fn
		28		B = 18 Fn
Paddy soils (3 samples) in Japan	25°C ± 0.5 aerobic (50% WHC)	10	Fumigation[e] method	B = 33 Fn
		28		B = 26 Fn

[a] B: Biomass-C, Fn: Flush of mineral N by $CHCl_3$ fumigation
[b] Ayanaba *et al.*[2] [c] Jenkinson *et al.*[8]. [d] Marumoto *et al.*[12]. [e] Jenkinson and Powlson[7]. [f] Anderson and Domsch[1].

Table 4. Percentages of the flush-N derived from dead microbial biomass and non-biomass after drying soils

Soil and location	Pretreatment	Incubation method		Flush mineralization	
				From biomass	From non-biomass
				%	
Upland soil[a] (1 sample) in W-Germany	Oven-drying (70°C, 24 h)	22°C ± 0.5 28 d., aerobic (34% of WHC)	(N)	76	24
Upland soils[b] (4 sample) in W-Germany	Air-drying (3 days, twice)	22°C ± 0.5 28 d., aerobic (34% of WHC)	(N)	55	45
	Oven-drying (70°C, 24 h)	22°C ± 0.5 28 d., aerobic (34% of WHC)	(N)	77	23
Paddy soils (samples *A, B, C*) in Japan	Oven-drying (70°C, 24 h)	25°C ± 0.5 28 d., aerobic (50% of WHC)	(N)	66	34

[a] Marumoto *et al.*[11] [b] Marumoto *et al.*[12]

N in microbial biomass contributes significantly to the larger amount of mobile N in paddy soils.

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Influence of plant roots on C and P metabolism in soil

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Key words C balance Exudates Microbial biomass Organic phosphates P-mobilization Phosphatase Priming effect Rhizodeposition Rhizosphere technique Root decomposition

Summary A technique for studying the modification of soil by plant roots is described. Using it, soil zones differently affected by plant roots can be separated for subsequent analysis. With this method, the transfer of C from roots of ^{14}C-labelled maize plants into soil and the change in soil C and P fractions were investigated.

The results show that the C released from roots to soil was 13% of the total assimilated C. The remaining root-derived C in soil was relatively small (15%). Maize roots induced a decrease in organic soil C and in both total and isotopically exchangeable soil P. On the other hand they increased the microbial biomass C, phosphatase activity, bicarbonate extractable organic P and phospholipid P and enhanced the incorporation of ^{32}P into organic P fractions. Both root C and root influences were detectable outside the immediate root zone.

These results demonstrate an intensive C turnover and P mobilization in the rhizosphere soil, including some organic P fractions, and suggest that the actual rhizosphere may be greater than is generally assumed.

Introduction

In recent years considerable progress has been achieved towards quantifying the transfer of C from plant roots to soil[18,22,23,29] using techniques for homogeneous ^{14}C labelling of plants growing in soil.

Helal and Sauerbeck[9] proposed a simple method for separating soil into three discrete zones differently affected by plant roots. Later, this technique was further modified to allow ^{14}C-labelling of the plants and sampling of rhizosphere gases and volatiles[11].

This paper describes the technique and some results obtained by its use in experiments dealing with C and P metabolism in soil as a function of proximity to roots.

Materials and methods

Fig. 1 shows the construction of a rectangular cell pot from plexiglass, having 3 soil zones separated by vertical stainless steel mesh plates (pore dia. 31.5 μm, open area 25%). The central zone is 20 mm wide with 10 mm intermediate and outer half-zone sections on either side. Each zone is filled with *ca.* 140 g air-dry 2 mm sieved soil and, after wetting to 65% W.H.C., 2 pregerminated maize seeds are planted in the central zone. Root hairs penetrate to the intermediate but not to the outer zone.

To follow the evolution of CO_2 from the soil, the root and shoot spheres are separated by the lid of the outer container. The plant shoots pass through appropriate openings of this

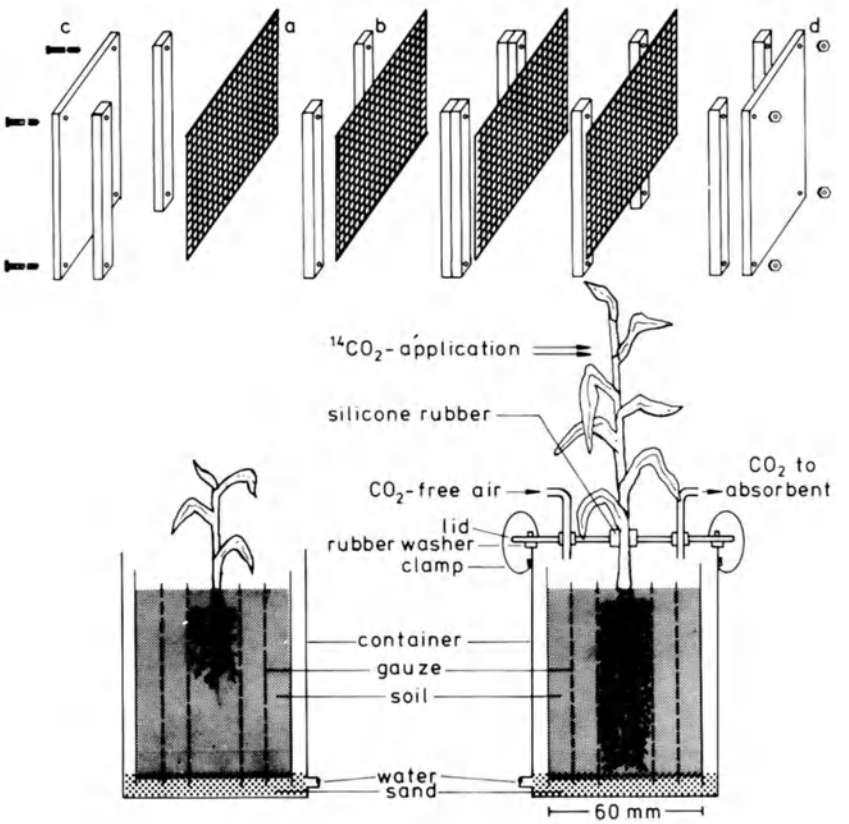

Fig. 1. Investigation of soil zones of different proximity to plant roots.

lid, the stems being sealed with silicone rubber. A circulating air stream allows continuous sampling of the soil air for analysis. At the end of an experiment the different soil zones are isolated by unscrewing the frame and all visible roots carefully removed. Further details and a discussion of practical advantages of this method relative to other comparable techniques of[3,8] were given by Helal and Sauerbeck[11].

In the present experiments *Zea mays* cv Garbo seedlings were grown in a chernozemic sandy loam soil: pH 7.5, organic C 1.5%, $CaCO_3$ 2.4%, organic P 229 $\mu g\,g^{-1}$ total P 250 $\mu g\,g^{-1}$. The growth conditions were: light intensity 25 $\times 10^3$ Lux, period 14 h, temperature 24°C day, 14°C night, R.H. 70%, CO_2 concentration 290–350 ppm.

Experiment 1: Assimilate consumption by roots and their influence on the C content of soil

Ten planted and four unplanted (control) pots were used. From day 8 to day 25 $^{14}CO_2$ (35 μ Ci/g C) was applied continuously to the plant shoots. $^{14}CO_2$ released from the soil (= root respiration and decomposition) was collected in 0.75 *M* NaOH for titrimetric determination and liquid scintillation counting. Further details of the long term labelling with $^{14}CO_2$ are described by Sauerbeck and Johnen[21]. On the 25th day plant and soil samples were analyzed for both total and labelled C in a Woesthoff-Carmhograph 5 and by liquid scintillation counting respectively. The inorganic C released from the soil by dilute acid in the presence of an antioxidant was also determined[25]: organic C was calculated by difference.

Experiment 2: Phosphatase activity and microbial biomass in soil

This was conducted in the same way as experiment 1, except that the plants were not

Table 1. Distribution of labelled C (per cent of total uptake) in maize plants and soil at day 25 after application of $^{14}CO_2$ to the shoots for 18 days

In shoots	In roots	Released from soil by root respiration and decay	Remaining in soil		
			In central zone	In intermediate zone	In outer zone
56	31	11	1.6	0.3	0.1

2 plants, 4.5 g d.m. per pot, 420 g soil, total ^{14}C-uptake 53 μCi – average of 10 replications.

Table 2. Deposition of maize root C (labelled) and change of soil C (unlabelled) in three soil zones at day 25

		Inorganic (mg C per 140 g soil)	Organic
Initial content		409	1943
Change in unplanted control		+ 5	− 32
Change in planted soil			
Central zone	soil-C	+ 46*	− 182*
	labelled C	+ 7	+ 17
Intermediate zone	soil-C	+ 11	− 58
	labelled C	trace	+ 5
Outer zone	soil-c	− 25	− 83*
	labelled C	trace	+ 2

* Significantly different from the control value ($P = 0.05$).

^{14}C-labelled. On the 25th day the soil zones were sampled for phosphatase assay and for $CHCl_3$ fumigation. The phosphatase activity was measured by the p-nitrophenyl phosphate assay procedure of Tabatabai and Bremner[26].

For the biomass determination, moist soil samples equivalent to 20 g dry soil (8 replicates) were stored for 5 days at 22°C to allow the effects of sampling to subside[20]. The $CHCl_3$ fumigation was carried out according to Jenkinson and Powlson[14]. After removing $CHCl_3$ the soil moisture was readjusted to 50% of the W.H.C. Both fumigated and unfumigated soil samples were then incubated for 10 days at 22°C in 1000 ml bottles each containing a vial holding 20 ml 1 *M* NaOH to absorb CO_2 which was subsequently determined by titration with HCl.

Experiment 3: Incorporation of ^{32}P into soil P

The soil was labelled with carrier-free ^{32}P (1.15 mCi/kg soil), wetted to 60% of the WHC and incubated at 40°C for 14 days. Unlabelled soil was also incubated under identical conditions for a determination of the isotopically exchangeable P. Afterwards the soil was air dried, ground to pass a 2 mm sieve, mixed thoroughly and filled into the mesh frames for planting.

After 25 days both the plant material and the samples from the different soil zones were analyzed for P content and ^{32}P-radioactivity. Various fractions of soil P were obtained by the following analytical methods: colorimetric determination[19]; isotopically exchangeable P[12]; total P ($HClO_4$ digest[5]); organic P (NaOH extract[24]); bicarbonate extractable P[4,6]; phytate P[7]; inositol[16]; and phospholipid P[15].

Results and discussion

Table 1 shows that 44% of the C assimilated by the plants was transferred to their roots of which *ca.* $\frac{1}{3}$ was released into the rhizosphere.

This result agrees with the findings of Martin[17]; Barber and Martin[2]; and Sauerbeck and Johnen[22] and demonstrates that even in early growth stages a significant part of the plant C is released by the roots. However, most of this is evolved as CO_2 by root respiration and decomposition while only a minor part remained in the soil mainly in the central root zone, but 25% of it was found beyond the central zone. Helal and Sauerbeck[10] proposed that volatile compounds may play a role in this organic C transport from the rhizosphere and in further experiments, not reported here, carbonyl and acid volatiles were detected in the air flushed from the root chamber.

Table 2 confirms that the incorporation of labelled root C is relatively small compared to the concomitant loss of soil organic C: this enhanced loss indicates a priming effect in the presence of roots.

The root C evolved in the rhizosphere as CO_2 is the sum of two components: the CO_2 originating directly from root respiration and that produced by the rhizosphere microorganisms metabolizing organic exudates released by roots. This organic C input is a significant source of energy for the rhizosphere organisms. Quantitative differentiation between root and microbial respiration is, however, experimentally difficult because of the possible influences of microorganisms on root metabolism[1,2].

Based on comparisons between sterile and nonsterile roots a 1:1 ratio for root respiration to microbial respiration has been proposed by Warembourg and Morrall[30]. Adopting this ratio and assuming that the $^{14}CO_2$ fixation in the rhizosphere is negligible, the total organic root C input in our experiment was $11/2 + 7 = 7.5\%$ of total assimilates (Table 1). Of this estimated amount 27% remained in the soil. Compared to known turnover rates of organic substrates in incubation experiments (Jenkinson and Ladd[13]), the apparent decomposition of exudates in this experiment seems to be relatively high but the reasons are not clear.

According to Table 3 the plant roots induced a considerable increase both in microbial biomass and in phosphatase activity, particularly in the central root zone, but this increase was still detectable in the outer soil layers. Unfortunately, the microbial biomass had not been determined in the earlier experiments, so the labelled C content of the microbial biomass is not known. However, the decrease in soil C (Table 2) and the simultaneous increase in biomass-C (Table 3) suggests that at least a part of this increase in microbial biomass in planted soil is due to the enhanced consumption of soil C.

There are some uncertainties with the estimation of microbial biomass in planted soils; one factor is the value of the mineralization

Table 3. Phosphatase activity and microbial biomass-C in soil zones differing in proximity to 25 d old maize roots

Soil zone	Phosphatase activity (10^{-8} moles g^{-1} h^{-1})	Biomass-C* (μg C g^{-1})
Control (unplanted)	22 (100)	207 (100)
Root zone	41 (186)	377 (182)
Intermediate	38 (172)	326 (157)
Outer zone	27 (123)	287 (138)
LSD, $p = 0.05$	11	76

* Based on a conventional mineralization coefficient $k_c = 0.411$

Table 4. Change in total and isotopically exchangeable P within differing soil zones

Soil zone	Total P (μg P g^{-1})	Isotopically exch. P (μg P g^{-1} soil)
Control (unplanted)	524	14.1
Root zone	477	5.8
Intermediate	517	12.9
Outer zone	521	12.7
LSD, $p = 0.05$	31	1.8

coefficient k_c. If bacteria were dominant in the rhizosphere[27] the real value of k_c should be lower than in normal soil. Consequently, the microbial biomass C in root affected soil zones may be even higher than the values given in Table 3.

Regarding soil P, Table 4 indicates that both total and isotopically exchangeable P decreased in the control root zone. However, the isotopically exchangeable P and its reduction is small relative to the decrease in total P showing that considerable mobilization of P occurred in the root zone from less available fractions.

The incorporation of ^{32}P into various P fractions (Table 5) showed a clear dependence on the presence of roots. The specific activity of the inorganic P decreased significantly compared to the unplanted control, presumably because of uptake by the plants. This indicates a depletion of the easily exchangeable P fractions. Contrary to this, the specific activities of the $NaHCO_3$-extractable organic P and phospholipid P increased close to the roots suggesting that some organic P fractions are turning over more rapidly near the roots.

This tendency to higher specific activities was observed with all organic P fractions. However, the identity of the "phytate" fraction is uncertain: ion exchange chromatography suggested the existence of inositol-containing P components but further examination failed to identify pure inositol hexaphosphate among them (Helal and Sauerbeck, in preparation).

The high phosphatase activity in the root zone (Table 3) suggests that the corresponding increases in organic P fractions are due either

Table 5. Distribution of ^{32}P in soil P fractions within differing soil zones

Soil zone	Inorg. P		NaOH-extr. org. P		$NaHCO_3$-extr. org. P		"Phytate"-p**		Phospholipid-P	
	$\mu g\ P\ g^{-1}$	s.a.*	$\mu g\ P\ g^{-1}$	s.a.	$\mu g\ P\ g^{-1}$	s.a.	$\mu g\ P\ g^{-1}$	s.a.	$\mu g\ P\ g^{-1}$	s.a.
Control	294	3.24	230	0.82	5.7	7.4	108	0.51	2.5	7.8
Root zone	267	2.88	210	1.16	12.0	9.3	116	0.78	4.9	9.6
Intermediate	301	2.89	216	1.19	8.3	7.4	110	0.83	3.8	9.1
Outer zone	300	2.98	221	1.09	6.6	7.1	112	0.70	2.9	8.3
LSD 0.05	21	0.30	23	0.14	1.5	1.6	22	0.25	1.4	1.6

* s.a. = specific activity $\mu Ci\ mg\ P^{-1}$

** isolated according to Cosgrove 1963

to an increase of microbial biomass P or to an enhanced extractability. Either or both explanations may be valid.

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Response of soil microflora to moorland reclamation for improved agriculture

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Key words Agricultural reclamation Exmoor Gley Microbial indices Microflora Pedogenic change Plate counts Podzol

Summary Major changes in the microbial populations, estimated by the dilution plate technique, of stagnohumic gley and thin pan stagnopodzol profiles occur as a result of moorland reclamation. There is an immediate 'surge' in bacteria as numbers increase by two orders of magnitude compared with unreclaimed soils. Bacteria dominated recently reclaimed soils and make up 97–99% of "total" counts more than one year after initial ploughing. Actinomycetes assume much increased importance in profiles reclaimed nearly 130 years and it is thought they adjust much more slowly to a new soil environment which leads to development of a brown earth topsoil. Evidence is presented which underlines the value of numerical estimates of microorganisms as indicators of soil ecological conditions and pathways of soil development.

Introduction

Agricultural reclamation in upland Britain has concentrated on increasing pasture production and livestock output in areas generally considered as climatically and edaphically 'marginal' for improved farming. Upland soils are commonly strongly podzolised or gleyed, frequently have highly acid and nutrient-deficient organic or peaty surface horizons and may include true peats which are anaerobic for much of their depth. Effective land improvement demands substantial changes in soil physical, chemical and biological properties which may in turn induce fundamental alterations in soil profile development. Thus Crompton[6] considered peaty surface horizons and the deep granular mull humus topsoil typical of a brown earth as reversible morphological forms depending on upland management regimes. Although Crompton could attach no precise time scales to such changes in northern England, recent work on Exmoor has indicated that A horizons with diagnostic brown earth characteristics can develop from stagnopodzol or stagnohumic gley surface horizons in less than 130 years[12,13]. Without providing supportive evidence Crompton attached considerable importance to the enhanced activity of microorganisms and other soil biota in bringing about major profile changes in the nature and organisation of soil organic matter, chemistry and

overall morphology. Much earlier, Waksman[17] had suggested that microbiological analysis of soil might provide a sensitive index of fertility and that "the relative number of bacteria, actinomycetes and fungi indicate also the chemical composition of the soil", Changes in the soil microflora resulting from moorland reclamation between 1845–48 and 1972–73 of a selection of well documented sites on Exmoor, SW England are examined for two main reasons: first, to define and interpret the character of the microbial response and second, to reassess the potential of soil microbial analysis as an indicator of changes relevant to soil fertility assessment.

Sites and methods

Two moorland soil types in central Exmoor have been investigated – a thin iron pan stagnopodzol mapped as the Burcombe Series and a stagnohumic gley identified as the Ashcombe Series[7]. Identical assemblages of surface peaty horizons are common to both profiles while lower horizons frequently demonstrate integrade features which suggest a close genetic relationship, a fact reinforced by the mosaic character of the majority of the mapping units. Type profiles, details of original vegetation cover, climatic characteristics, geology and physiographic details for Exmoor are given by Curtis[7] and Maltby[13].

Reclamation practices

Nineteenth century reclamation involved (i) paring-off 5–7.5 cm of turf (ii) burning the dried turf stacks (iii) spreading residual mineral ash (iv) ploughing and cultivation including subsoiling of peaty soils (iv) application of carbonate lime (usually 6.3–7.6 t/ha) and (vi) reseeding with varied mixtures but usually including *Holcus lanatus, Cynosaurus cristatus, Dactylis glomerata* and *Phleum pratense.* Modern schemes now omit treatments (i) and (iii), include the addition of N and/or combination inorganic fertilizers and concentrate on white clover–ryegrass varieties. The depth of recent ploughing is generally 20–30 cm and subsequent cultivation modifies the surface 5–10 cm.

Sites and sampling procedures

The three sites listed below were selected where time since first ploughing varied from one week to nearly 130 years. All occupy south-facing slopes within an altitudinal range of 460–520 m and on gradients of 1–5°. In each case adjacent moorland areas or strips within the scheme were left intact to be used as controls in a 'paired' sampling strategy.

Sampling for microbiological analysis was based on 2 cm depth bands largely within morphologically distinct organic soil horizons. The 3–5 cm band was consistently sampled to establish a reference position in the main rooting zone among profiles and to compare treatment effects independent of subsequent horizon differentiation. Further soil analyses were carried out on the microbial samples themselves, on additional successive 2 cm samples and in some cases bulk horizon material.

1. Goat Hill (SS726405)* Reclamation in June 1974 extended over *stagnohumic gley, thin iron pan stagnopodzol and integrade profiles* which had been sampled previously[12]. Hitherto stagnohumic gley profiles were sampled at the completion of cultivation one week after initial ploughing before any chemical amendment, and samples were also taken simultaneously from a comparable profile on the immediately adjacent moorland.

2. Red Deer (SS805396) Reclamation of principally thin iron pan stagnopodzols occurred

* UK National Grid Reference Number

between 1972 and 1973. Profiles were examined nine months after cultivation, application of carbonate lime and basic slag (5.0 t and 1.2 t/ha respectively) and reseeding. In addition 42 samples were taken from the 3–5 cm band only, on six dates May–October 1973 representing conditions 10–15 months after reclamation. 19 additional 3–5 cm samples were collected in nine sampling visits to unreclaimed blocks during April 1973–May 1974. Samples were taken from treated and control areas simultaneously or at overlapping dates to maintain acceptable control over non-synchronous variations due to season or weather.

3. Pinkery (SS725415) An area of mainly stagnohumic gley and intergrade profiles was reclaimed in 1845–48 reploughed and reseeded in the early 1940's and again in 1976. Carbonate lime and basic slag was applied on both occasions and also in 1966 at a rate of 5.0 t and 1.25 t/ha respectively. A significant part of the area has reverted towards the pre-reclamation condition as indicated by the high proportion of moorland plants (*e.g. Juncus squarrous* and *Molinia caerulea*) in the sward, infestation by *Juncus effusus* and accumulation of a distinct surface root mat and litter layer. Profiles from unreclaimed, reclaimed and reverted areas were sampled simultaneously at various dates 1972–74. These were supplemented by additional 3–5 cm samples from the reclaimed and unreclaimed strata.

Methods

Enumeration of microflora The numbers of four groups of microorganisms: bacteria (B), actinomycetes (A), filamentous fungi (F) and yeasts (Y) were estimated using the soil-dilution plate method developed initially by Waksman[18]. Bacteria and actinomycetes were counted on Jensen's medium[11] modified by the addition of cycloheximide at 4 mg/100 ml to suppress fungal interference[4]. Martin's Rose Bengal-Streptomycin medium[15] was used for the enumeration of filamentous fungi and yeasts. Population estimates, therefore, are based on colony forming units (CFU's) which may be derived from all types of viable microbial propagule. Bulk samples were placed in a thermally insulated box for transport to the laboratory before plating, generally three to six hours after collection. In several cases samples were stored up to 12 hours at 4°C. All results were calculated on a soil volume basis because of differences in bulk density between samples and horizons. A detailed discussion of the analytical advantages and limitations, methodological problems and precise specifications of procedures are given by Maltby[13].

Soil analyses Methods used for the analysis of additional soil properties and data obtained are recorded elsewhere[13].

Results

Figures 1 and 2 show the distribution of the four microbial groups, pH values and horizon morphology for profiles sampled from unreclaimed, reclaimed and reverted areas at the three sites.

Response in the stagnohumic gley one week after cultivation: Site 1 (20/6/74)

Estimates of 'total' CFU's from Op material exceed by up to two orders of magnitude figures obtained at comparable depths from the Oh1 horizon in the unreclaimed profile (Fig. 1.1). The immediate effect of reclamation is not restricted to the surface horizon. Whilst the former 0–2 and 3–5 cm levels buried in the ploughed profile

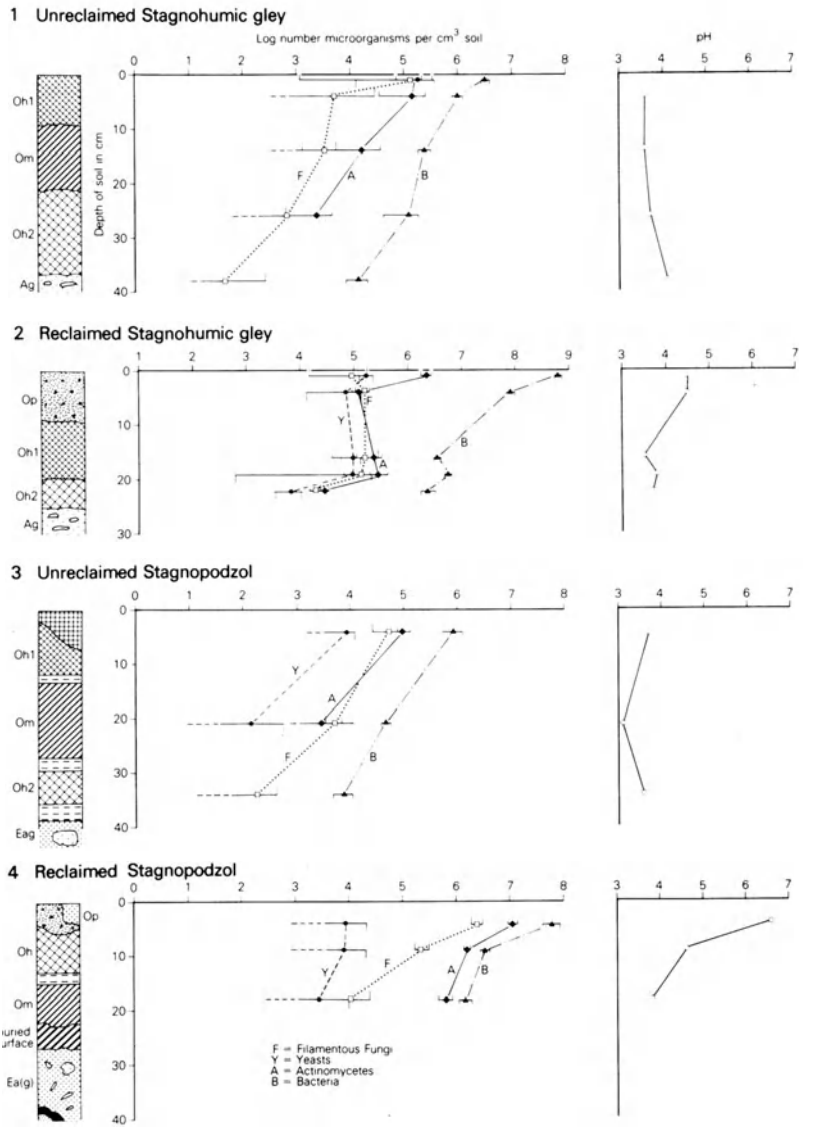

Fig. 1. Microbial profiles and analytical characteristics of (1) Unreclaimed stagnohumic gley Goat Hill, (20/6/74). (2) Reclaimed stagnohumic gley Goat Hill one week after initial cultivation, (20/6/74). (3) Unreclaimed thin iron pan stagnopodzol Red Deer, (24/5/73). (4) Reclaimed thin iron pan stagnopodzol Red Deer nine months after cultivation, (31/5/73). Horizontal bars indicate 95% confidence interval.

exhibit only slightly higher 'total' estimates than those from the unreclaimed profile there is a very marked increase in the unploughed basal peat (Oh2) of the reclaimed soil (Fig. 1.2).

Fig. 1.2 also shows that bacteria proliferated far more than the other groups, the increase at 0–2 cm being from $3.52 \times 10^6/cm^3$ in the intact

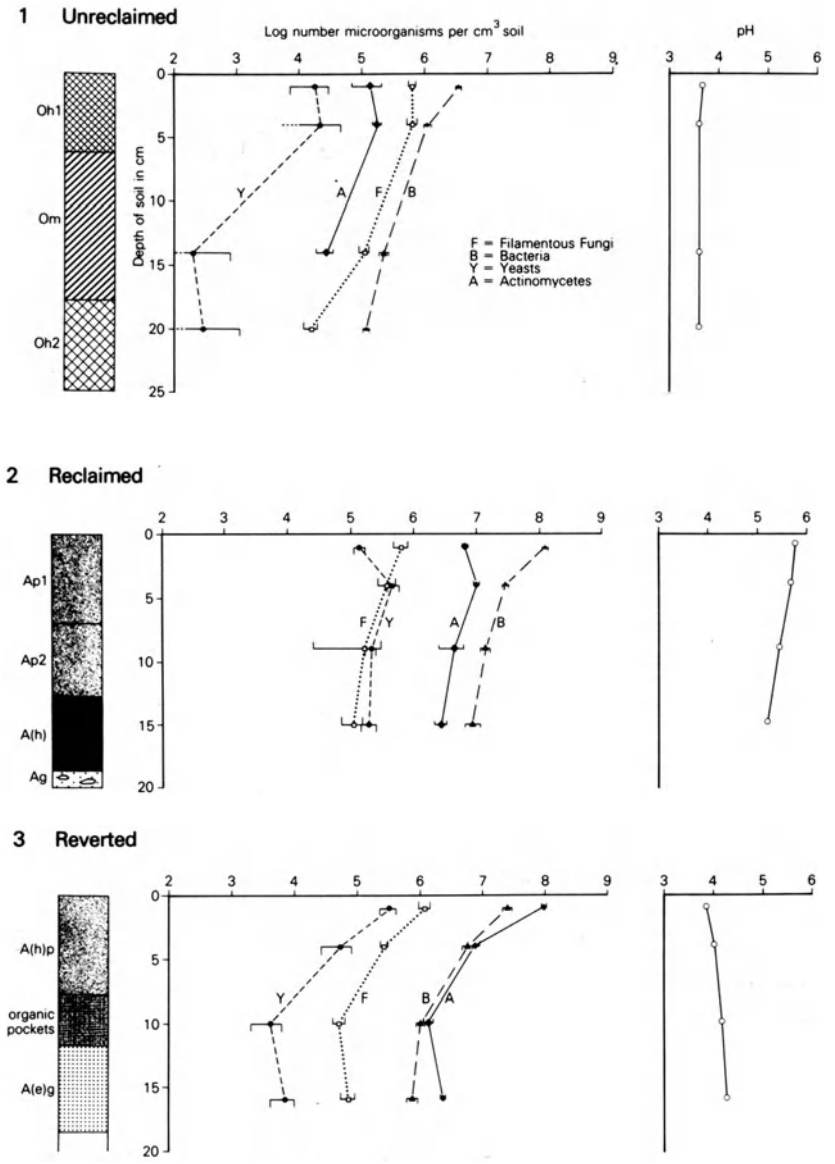

Fig. 2. Microbial profiles and associated soil properties in unreclaimed, reclaimed (1845–48) and reverted areas of the stagnohumic gley mapping unit, Pinkery, (20/11/73). Horizontal bars indicate 95% confidence interval.

profile to $645.02 \times 10^6/cm^3$ soil in the reclaimed profile. They contribute $> 99.5\%$ of the count in the Op samples. However, significant variations occur within the new Op horizon over very short vertical sampling distances and this is an important and general feature of all recently reclaimed profiles (Maltby, 1977). pH increased slightly without liming from below 4 to 4.5 in the plough layer.

The similarity of response among the different microbial groups is strikingly illustrated in the similar shapes of the reclaimed log-plots which clearly show the original surface now buried at 20 cm. The pattern seems to point to the influence of broad environmental controls rather than specific factors which might differentially influence particular groups.

Response in the thin iron pan stagnopodzol nine months after cultivation: Site 2 (24 to 31/5/73)

The difference in 'total' CFU's between unreclaimed (Fig. 1.3) and reclaimed (Fig. 1.4) profiles at 3–5 cm nearly two orders of magnitude. Bacteria, actinomycetes and filamentous fungi are all significantly higher in this band but yeasts exhibit no significant change. As at Goat Hill the effect of reclamation is not restricted to the comminuted Op horizon and very significant increases especially of bacteria and actinomycetes occur in inverted but otherwise still intact peat horizons. This results in a greater decline in numbers with depth between the Oh and Om horizons of the reclaimed profile (Fig. 1.4). The most obvious contrast with the pattern in the stagnohumic gley at Site 1 immediately after cultivation is the marked increase in actinomycetes which is clearly underlined by a change in A:B ratios and proportional representation of this group in the counts[13]. Lime and basic slag have produced a very large increase in exchangeable Ca and in pH which exceeds 6 in the new Op horizon.

Stagnohumic gley profiles 126–129 years after reclamation: Site 3

Reclaimed profiles typically demonstrate large increases in bacteria, actinomycetes and yeasts (Fig. 2). Relatively high numbers of actinomycetes produce consistent increases in the A:B ratio and their proportional representation over the unreclaimed soil in all but the surface sample. However, the group assumes even greater significance in the reverted soil where actinomycetes are numerically dominant at all levels sampled. An A:B ratio at 0–2 cm of 3.80 compared with values of 0.05 in the reclaimed profiles and 0.04 in the unreclaimed peaty surface marks a major ecological transformation. Yeast counts also have responded to the new environmental conditions associated with reclamation. The subsurface population in the reverted profile has fallen generally to a position intermediate between the unreclaimed and reclaimed pattern.

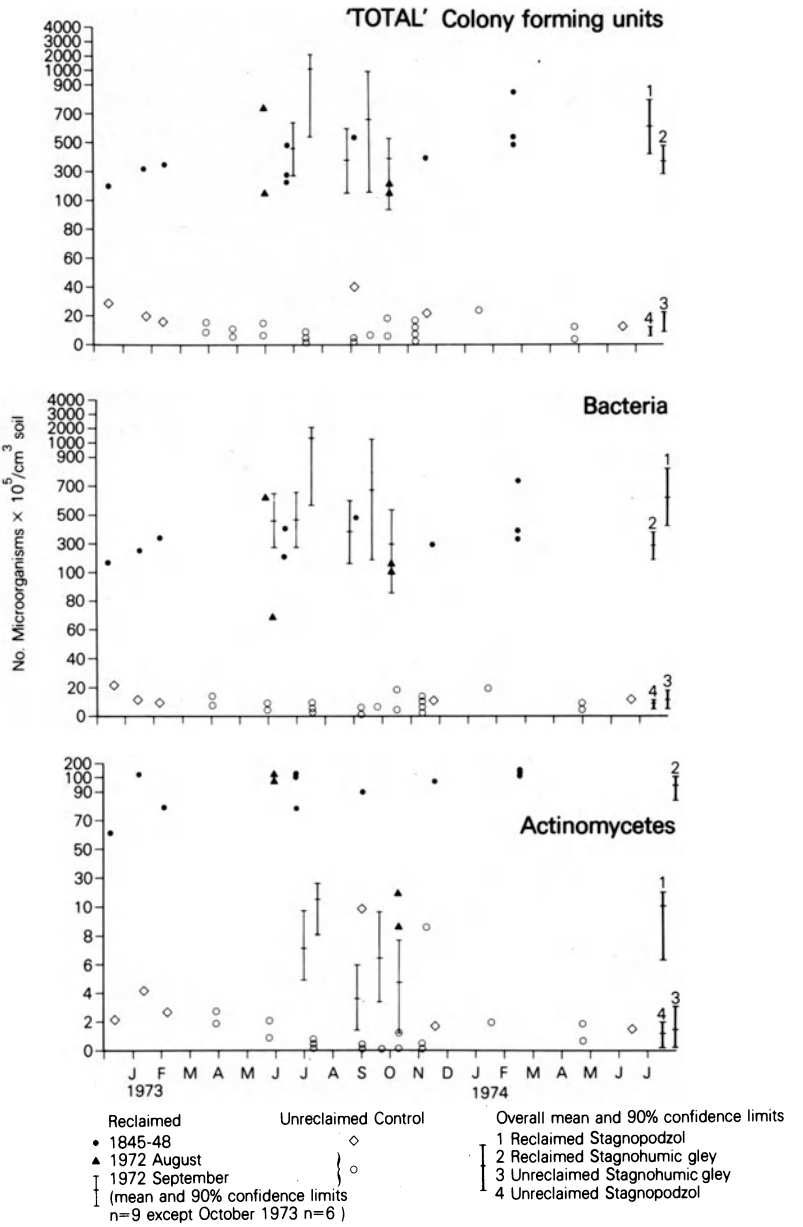

Fig. 3. Seasonal variations in CFU's at 3–5 cm in unreclaimed and reclaimed strata of stagnohumic gley and thin iron pan stagnopodzol profiles, Exmoor 1972–73.

Overall comparison of microbial estimates between unreclaimed and reclaimed soils at 3–5 cm

The results of 'total' counts and of enumeration of bacteria and actinomycetes for the three sites are summarized in Fig. 3.

Any seasonal or environmentally-induced temporal variation in

total CFU's is considerably less than the differences caused by reclamation (Fig. 3). Overall, there is no statistically significant difference in total CFU's between the stagnohumic gley and stagnopodzol in either their unreclaimed or relcaimed phase ($p < 0.05$). However, there is a very clear separation between the unreclaimed and reclaimed condition of each soil. Examination by groups indicates some interesting contrasts between the recently reclaimed stagnopodzol and long improved stagnohumic gley. Bacteria are significantly higher ($p < 0.10$) in the stagnopodzol where they constitute 97% of the total estimate compared with only 74% in the case of the stagnohumic gley. However, actinomycetes are much more prominent in the long reclaimed stagnohumic gley. They comprise nearly 25% of CFU's compared with just 2% in the case of stagnopodzol.

Discussion

Bacteria comprise on average 72% of total CFU's at 3–5 cm in the root mat peat (Oh) of the unreclaimed thin iron pan stagnopodzol and 63% in the case of stagnohumic gley. These observations confirm the predominant numerical representation of bacteria in acidic soils discussed by Holding *et al.*[10] and detected by many others *e.g.* Corke and Chase[5] and Rybalkins and Kononenko[16]. The proportional representation of groups indicate, despite slightly higher filamentous fungi estimates in the gley, an overall similarity in the microbial spectrum between the two soil types at 3–5 cm. Thus the F:Y ratio is 2.66 in the podzol and 2.57 in the gley. Respective A:B ratios of 0.18 and 0.16 confirm comparability between morphologically and analytically inseparable O horizons despite their association with pedologically different lower mineral horizons.

Seasonal variations are small in comparison with differences in estimates between sampling depths in the profile or between reclamation treatment. No attempt is made to explain in any detail the microbial spectrum of the unreclaimed condition. However, the consistently low numbers are a clear reflection of a highly acidic (pH mainly 3.5–4.0), frequently waterlogged, anaerobic, nutrient-poor soil environment which must pose severe restrictions on microbial activities and population development.

Reclamation brings about an immediate microbial response best defined as a bacteria 'surge' after Cook[3] who used the term to describe soil microbial changes when organic residues were incorporated by ploughing. Burges[2] has suggested that ploughing increases bacteria populations by 20–30 times whilst actinomycetes and fungi usually increases by only 2–3 times. The surge at Goat Hill indicates that

for the bacteria at least this may be a gross underestimate of the scale of microbial change when moorland soils are first ploughed. As early as 1909 Doryland and King[8] had demonstrated the microbial effects of deep ploughing linked with increased ammonia production and a decrease in denitrification. In the case of the reclaimed stagnohumic gley improved aeration, release of CO_2 and perhaps most important sudden availability of large amounts of damaged plant tissue and other organic substrates are all likely contributing factors. However not only the scale but also the speed of response is a marked feature of the transformation. The immediate increase in proportional representation of bacteria to more than 99% at a number of profile sampling levels at Goat Hill and their 97% contribution at 3–5 cm in the reclaimed stagnopodzol at Red Deer 9–15 months after initial cultivation underlines a major alteration in the microbial spectrum. The increase below the level of ploughing emphasises the importance of a general aeration effect on the entire organic part of the profile.

The microbial response to reclamation is complex. The reclaimed soil initially is physically, chemically and biologically heterogeneous due to the special variability of mechanical mixing, liming, early plant cover and root development and the manuring effect of grazing animals. Generally higher coefficients of variation characterise the recently reclaimed stagnopodzol (1.0 for 'total' counts and 1.02 for bacteria) in comparison with the unreclaimed condition (0.62 for 'total' counts and 0.68 for bacteria).

Total CFU's at 3–5 cm in the recently reclaimed profiles are 60% higher than the estimate for stagnohumic gleys and intergrades reclaimed in the nineteenth century. The influence of the initial surge still seems clear more than a year after cultivation. However, with time a new and contrasting microbial profile emerges. Bacteria decline in absolute and relative terms but more impressively actinomycetes increase to comprise 25% of the counts. Filamentous fungi and yeasts become very minor numerical components of the microflora at 3–5 cm. The result is an increase in A : B and a reduction in F : Y compared with unreclaimed and recently reclaimed conditions. Detailed patterns of counts and their ecological interpretation have been discussed by Maltby[12].

Actinomycetes characteristically are relatively slow growing organisms, but include species capable of breaking down highly complex organic substrates such as might be expected to predominate in humified peat[9]. They are not generally thought capable of growing in waterlogged soils and although abundant evidence exists for acidophilous types[4,19] they are considered ineffective substrate colonisers if pH

falls below 5. There is good reason to suppose that this group adapts more slowly to new soil environmental conditions but that over time they may be extremely important in the gradual mineralisation of residual peaty substrates and contribute to the development of the brown earth-like A horizon which characterises the reclaimed profile. The relatively homogeneous horizon has developed incongruously above gleyed, podzolised or intergrade mineral horizons. It is typified by improved drainage and aeration, an even distribution of organic matter intimately mixed with the mineral fabric, by relatively high and even distribution of mineral nutrients and pH.

The distribution of pyrophosphate and dithionite extractable forms of amorphous iron[1] recorded by Maltby[13] confirms that the morphological brown earth features developed in the 130 year old reclaimed soil correspond with the microbial changes and increase in soil fertility which allows the growth of improved swards and increased stocking rates. The pedogenic change is associated with an estimated loss after initial paring and burning of 7 kg/m^2 of organic C^{14} and consequent changes in the spectrum of different microbial groups revealed by the soil dilution plate method.

Sensitivity of the biotic approach is underlined by the subtle change which occurs as a result of revertence. Total numbers fall, actinomycetes assume dominance over bacteria and F:Y ratios increase markedly as the soil becomes more acid, organic substrates more complex and nutrient poor and a N-deficient litter layer more prominent under a reduced grazing regime. The inevitable consequence of continued organic matter accumulation would be the development of a peaty horizon and enhancement of gleying or podzolisation characteristics. Reduction in microbial numbers suggested by the fall in CFU's would be compatible with such a trend towards the original pre-reclamation profile.

Conclusion

Significant changes in the soil microflora occur when moorland soils are reclaimed for improved agriculture. The soil dilution plate method appears to give estimates of microbial groups which can be interpreted meaningfully in the light of major soil ecological and pedogenic transformations and suggests the microflora to be a sensitive measure of soil conditions. The ideas of Waksman would thus seem worthy of serious re-evaluation. The precise capabilities of elements of the microflora in directing and determining the rate of soil changes have yet to be elucidated but would merit further in-

vestigation especially in the longer term manipulation of moorland soil fertility.

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Section 4

Anaerobic processes in soils and gaseous losses of nitrogen

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Anaerobic processes in soil

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Key words Aggregate oxygen profile Anaerobic radii Anaerobiosis Anoxia Aromatic ring cleavage Clostridium Dechlorination Denitrification Denitrification pathway hypothesis Fermentation Fe^{3+} Mn^{4+} reduction Methanogenic microorganisms ^{13}N ^{15}N N–N bond N_2O NO_3^- respiration/dissimilatory reduction O_2 consumption O_2 diffusion coefficient O_2 micro electrode Pesticides Reduction NO_3^- NO_2^- SO_4^{2-} CO_2 H^+ Soils flooded poorly and well drained Sulphate Xenobiotics

Summary Anaerobic conditions in soil affect plant productivity as well as organic matter and nutrient dynamics. Anaerobic processes often dominate biological and chemical features of flooded and poorly drained soils but in well-drained soils, anoxia is restricted to small zones and to limited periods. The anaerobic processes listed according to their approximate sequence of occurrence as the redox decreases are: Fe^{3+} and Mn^{4+} reduction, denitrification, fermentation, nitrate respiration, dissimilatory nitrate reduction to ammonium, sulfate reduction, carbon dioxide reduction, acetate splitting, and proton reduction. Two of the anaerobic processes, denitrification and fermentation of pollutant chemicals have been studied and recent results are summarized here. We describe the measurement of denitrification using a recirculating atmosphere of acetylene, provide quantitative information on the effect of moisture and oxygen on denitrification, and report on the oxygen concentration within soil aggregates measured by oxygen microelectrodes. The current hypotheses for the pathway leading from nitrate to the N–N bond and N_2O are also presented. Recent work in our laboratory has shown a new reaction for the metabolism of some chlorinated organic chemicals. In this reductive dehalogenation, the ring Cl is replaced by a proton. An unusual organism which carried out this reaction has been enriched and isolated on 3-chlorobenzoate. These anaerobic reactions may be of further use in pollutant removal. With these new techniques and knowledge available, it is now possible to gain a better understanding of the biochemistry, physiology, ecology and diversity of the anaerobic organisms and their processes in soil.

Introduction

Most cultivated soils can be considered as being dominated by aerobic conditions, but there are situations when anaerobiosis prevails which can be harmful to plant productivity. Detrimental effects of anaerobiosis include roots stressed by anoxia; loss of N by denitrification; production of organic acids, H_2S, and perhaps other plant toxicants; and favored conditions for the development of root pathogens. But, not all effects of anaerobic conditions are detrimental. Beneficial aspects of anaerobiosis are: N_2 fixation by free-living heterotrophs is usually more significant under anaerobic conditions; mineralization of organic matter is retarded; certain pesticides, *e.g.* DDT^{19}

and lindane, are more rapidly degraded; and anaerobiosis can be effective in control of certain plant pests.

Anaerobiosis occurs in soil when the oxygen consumption rate exceeds the supply rate. The rate of oxygen consumption depends primarily on the amount of available carbon for respiration with its rate of use being regulated by water availability and temperature. The oxygen supply rate depends on the moisture content and the physical characteristics of the soil, especially porosity which is influenced by structure and texture. A change in either the rate of oxygen consumption or supply can bring about anaerobiosis, *e.g.* a large application of manure or by compaction of the soil. Most soil anaerobiosis is caused by high soil moisture resulting from a high watertable or heavy rains. The dramatic effect of water on the aeration status is due to several factors: the much lower oxygen diffusion coefficient of water filled pores than of air filled pores, a much smaller reservoir of oxygen in soils with a high proportion of water filled pores, and a stimulation of respiration caused by solubilization and movement of nutrients and resurgence of metabolism after dessication.

Soils exhibit a continuum from those that are flooded and continuously anaerobic to the sandy, excessively drained soils for which it is hard to imagine any anaerobic microsites. For simplicity, however, it is reasonable to consider three major classes: the flooded soil (*e.g.* paddy rice culture), the poorly drained soils, and the well-drained soils. The flooded soils will have a few to several millimeters of aerobic soil overlaying deeper soil that remains anaerobic for the duration of flooding[6]. In this situation it is expected that all anaerobic microbial processes can occur. The poorly drained soils have significant periods of anoxia caused by a high watertable in the spring and following heavy rainfalls when temporary anaerobic sites are created. The well drained soils may have limited areas and periods of anaerobiosis; most likely when there are large, water saturated aggregates but it is unlikely that all the anaerobic processes occur, at least to any significant extent.

Anaerobic microorganisms and processes

The anaerobic microbial processes are summarily listed in Table 1 according to their approximate sequence of occurrence as the redox of soil decreases. The first four processes are those which might be expected in any soil with temporary anaerobic microsites, *e.g.* well-drained soils. The organisms that carry out these processes are all facultative anaerobes and thus easily make the shift from aerobic to

Table 1. Anaerobic microbial processes and their reaction products

Process	Reaction[a]
Fe^{3+}, Mn^{4+} reduction	$OM^{b} + Fe^{3+}, Mn^{4+} \longrightarrow Fe^{2+}, Mn^{2+}$
Denitrification	$OM + NO_3^- \longrightarrow N_2O, N_2$
Fermentation	OM $\longrightarrow$ organic acids, principally acetate and butyrate
Nitrate respiration	$OM + NO_3^- \longrightarrow NO_2^-$
Dissimilatory NO_3^- reduction to NH_4^+	$OM + NO_3^- \longrightarrow NH_4^+$
Sulfate reduction	OM or $H_2 + SO_4^{2-} \longrightarrow S^{2-}$
Carbon dioxide reduction	$H_2 + CO_2 \longrightarrow CH_4$, acetate
Acetate splitting	Acetate $\longrightarrow CO_2 + CH_4$
Proton reduction	Fatty acids and alcohols + $H^+ \longrightarrow H_2$ + acetate + CO_2

[a] The major reduction products are shown. Oxidized products are also produced; this is usually CO_2 if the electron donor is an organic compound.
[b] OM = Organic matter.

anaerobic growth. The first process, Fe^{3+} and Mn^{4+} reduction, is the only one for which no specific enzyme is responsible; these reductions are thought to result indirectly from other microbial products. The dissimilatory reduction of NO_3^- to NH_4^+ is carried out by both facultative and obligate anaerobes but this process is not prevalent in most soils probably because the carbon to electron acceptor ratio of soils is not sufficiently high to select for this reduction[35]. The last four processes are carried out by obligate anaerobes that are not expected to be significant in soils except under flooded or intensively anaerobic conditions. The last three processes in Table 1 occur when organic matter is converted to methane by lithotrophic bacteria that reduce CO_2 with H_2 to form methane, and by bacteria that split acetate into CH_4 and CO_2[2,28]. Proton reduction is carried out by acetogens that anaerobically oxidize butyrate and propionate to acetate[28]. These are the only known organisms that can utilize these organic acids in the absence of external electron acceptors. If methane is produced, usually these acetogens are also active: if not, the increased acidity would inhibit methanogenesis.

Organisms capable of anaerobic growth in soil include the facultative and obligate anaerobes. According to Skinner[19] the former are quite numerous representing up to 10% of the total soil population (*i.e.* 10^7 organisms per g soil) detected by plate count. It is the facultative anaerobes that are probably responsible for fermentation in the soils with the more temporary anaerobic conditions. The Clostridia are the most numerous obligate anaerobes and exist in densities of 10^3 to 10^5 organisms per g soil. Clostridial strains in soil can ferment carbohydrates (saccharolytic), proteins (proteolytic), hydrolyzed

cellulose, and fix N_2. It is thought that in dry soil Clostridia mostly exist as spores rather than as vegetative cells so they are generally inactive and slow to respond when conditions change. In poorly drained and flooded soils, they would be expected to play a more important role than in the more aerobic soils.

Soil scientists who do research with air dried, stored soils should be aware that this treatment favors spore forming organisms like Clostridia. When water is added to these dry soils, spore formers would be expected to have a proportionally greater impact on the soil metabolism than they would under field conditions.

Sulfate-reducing bacteria and methanogens are the other obligate anaerobes known to exist in soil, but soil is not considered a favored environment for their growth. Whether other obligate anaerobes exist in soil has not been investigated. Recent advances in anaerobic technique plus new interest in these little studied organisms has revealed a large number of new genera of anerobic heterotrophs, sulfate-reducing bacteria, and methanogens[2,9,28]. Whether any of these newly discovered organisms also exist in soil is not known, but there is no evidence that other obligate anaerobes are of practical importance in soil.

Microorganisms are considered anaerobic when they have a mechanism to generate energy (grow) other than by coupling electron transport to oxygen reduction. However, some anaerobes are also inhibited by oxygen; these are the obligate anaerobes. The reason for this inhibition is thought to be the production of toxic oxygen forms such as singlet oxygen (anti-parallel spin of the outer pair of electrons) and the reactive forms resulting from one electron reductions of oxygen illustrated by the reductive sequence shown in Fig. 1. These forms can occur during the respiratory reduction of oxygen and also from oxygen reaction with flavins, thiols, and other cell constituents. Aerobic organisms have one or more of the enzymes shown in Fig. 1 to remove these toxic intermediates while anaerobic organisms usually do not. However, for these toxic intermediates to be produced, there must be a flow of electrons. In soil where metabolism is severely restricted by the lack of available C, there may not be sufficient electron flow to generate these toxic intermediates. This may help explain why very oxygen sensitive organisms, such as methanogens, can survive in "aerobic" soil. Recently Kiener and Leisinger[10] showed that several methanogens were less harmed by oxygen when in the starved condition[10].

The presence of obligate anaerobes in a soil cannot be used to indicate the prevalence of anaerobic conditions since all three groups – Clostridia, sulfate-reducing bacteria, and methanogens – can survive

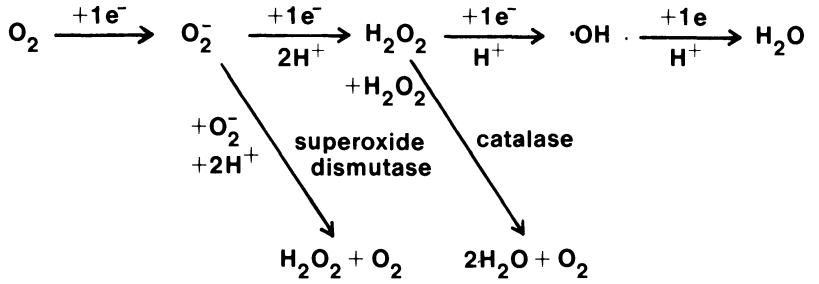

Fig. 1. The toxic intermediates formed from single electron reductions of O_2. The enzymes which protect aerobic organisms from these toxicants are also shown. Anaerobes often lack these protective enzymes.

well in the absence of anaerobic growth conditions. How long they can survive and how often they must experience anoxia to maintain their population is not known. It does seem, however, that the obligate anaerobes in soil are present in low numbers and show limited diversity relative to the known organisms capable of anaerobic growth, which supports the concept that most non-flooded soils are generally aerobic. Hence, the anaerobic processes that most likely occur in cultivated soils are those carried out by facultative anaerobes, the first four processes in Table 1. Of these, Fe^{3+}, Mn^{4+} reduction, denitrification and NO_3 respiration require the aerobic generation of the oxidized ions first which suggests that these processes should be more significant wherever there are more aerobic-anaerobic interfaces. Fig. 2 illustrates this interface. The anaerobic front in the aggregate is thought to expand and contract in response to the supply and respiratory consumption of oxygen. This concept suggests that denitrification, for example, should be more extensive in habitats where the area of the interface is greatest and where the movement of this zone is periodic.

Of the anaerobic processes in soil, denitrification and fermentation are of most practical importance and are further illustrated here with a summary of the current work from my laboratory. For complete information the reader should refer to our detailed papers by Parkin *et al.*[11,12,13], Sextone *et al.*[17,18] and Parkin and Tiedje[14].

Methods to study denitrification in soil

Denitrification has been a very difficult process to study because of the inability to measure the major product (N_2) in the atmosphere. We have used ^{13}N, ^{15}N, and the acetylene inhibition of N_2O reduction[3,24,27] to study denitrification since each method has unique advantages. ^{13}N, the longest lived radioactive isotope of nitrogen (half-life 9.96 min), is the only method where one can directly measure

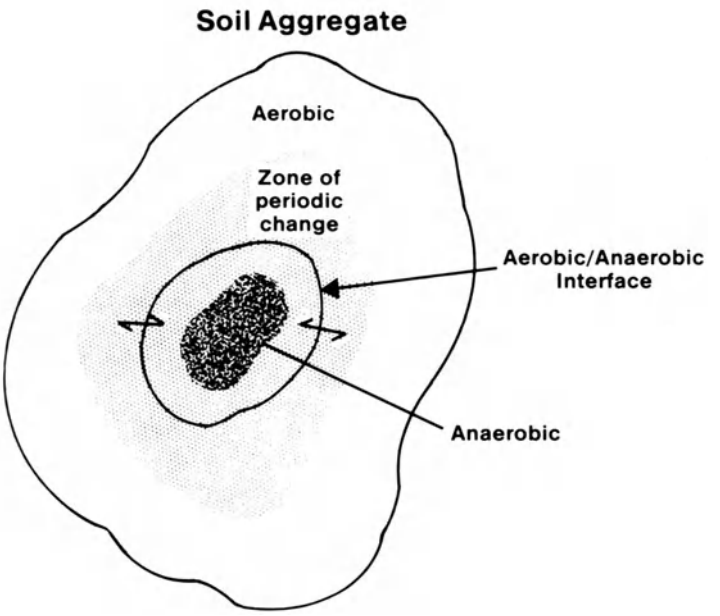

Fig. 2. Diagram of oxygen status of soil aggregate which illustrates how the anaerobic zone expands and contracts periodically in response to moisture changes. It is suggested that this dynamic movement is important for processes that require both aerobic and anaerobic conditions, *e.g.* denitrification.

the denitrification product in the natural atmosphere. Furthermore, ^{13}N allows very sensitive measurements (approximately 10^9 orders of magnitude more sensitive than ^{15}N for short-term measurements), and, because of this sensitivity, one can make labelled N additions that are well below the Km of the process thereby not affecting the natural rate. Use of ^{15}N is best suited for N balance experiments in the field where many samples are required and one wants to integrate information over time. The acetylene inhibition method is sensitive, can be used in the field, and, very important, relies on the natural NO_3 substrate concentration and distribution, something not possible by any label method. We believe that the acetylene method offers the most promise as a routine method to study denitrification in laboratory and field because of the number of samples that can be analyzed and the fewer assumptions required in interpreting the results. Acetylene is known to have other effects but the only one of significance to short-term studies of denitrification is the inhibition of nitrification[8,26] *if* the NO_3 concentration is very low.

We have developed a system using intact soil cores to measure natural rates of denitrification in which acetylene is distributed by gas flow. This method is more thoroughly described and evaluated

in Parkin *et al.*[11]. Briefly, acetylene plus oxygen at the concentration measured in the soil pores[14] is recycled through soil by means of a membrane pump. The rate of N_2O production is measured by electron capture gas chromatography, and, when this production is linear, it should reflect the rate of denitrification. The basic advantage of this acetylene method is that the forced air flow overcome the slow diffusion of acetylene into and N_2O out of the soil. Denitrification rate measurements can be made within 1 to 2 hours and thus the rate should reflect the soil conditions in the field before the sampled core has been significantly influenced by its removal from the environment. We have automated this method so that eight cores can be run simultaneously with up to 60 cores processed per day. Denitrification losses measured by this method were compared to ^{15}N losses determined by difference for cylinder microplots at two field sites. The losses determined by the acetylene method were 1.5 to 3 times less than the ^{15}N loss estimates although the 95% confidence intervals overlapped. This difference between the methods could be due to the higher moisture content of the soil in the ^{15}N microplots, to the inaccuracies and assumptions associated with measurement of total denitrification by ^{15}N, or to a greater proportion of aerobic microsites that might be caused by the forced gas flow. Our evaluations to date, however, have not shown a significant inhibitory effect of the forced air flow on denitrification[11]. Our experience with the ^{15}N difference method is that it is subject to more inaccuracies than the acetylene core method. The problems result from the difficulty of obtaining and maintaining a uniform label distribution; obtaining accurate, time-dependent measurements of ^{15}N ratios of the various N pools: and the required assumptions on the various fates and recycling of N.

The effect of oxygen and moisture on denitrification

We have used the acetylene recirculation method to measure the effect of oxygen concentration on denitrification (Fig. 3). The data on denitrification rate (circles) are expressed as a percentage of the anaerobic rate (using argon as recirculation gas) of the measured core, to normalize to a standard condition and to reduce the variability among cores[14]. The solid line in the figure was generated from the model of Smith[20] which predicts anaerobic volume of aggregates as a function of aggregate size, respiration rate (Q), pore space oxygen concentration, and the oxygen diffusion coefficient (D). The figure also shows the values of Q and D used to obtain this best fit line. Although we have not yet measured these values, the similarity in shape of the denitrification rate and the anaerobic volume as a function

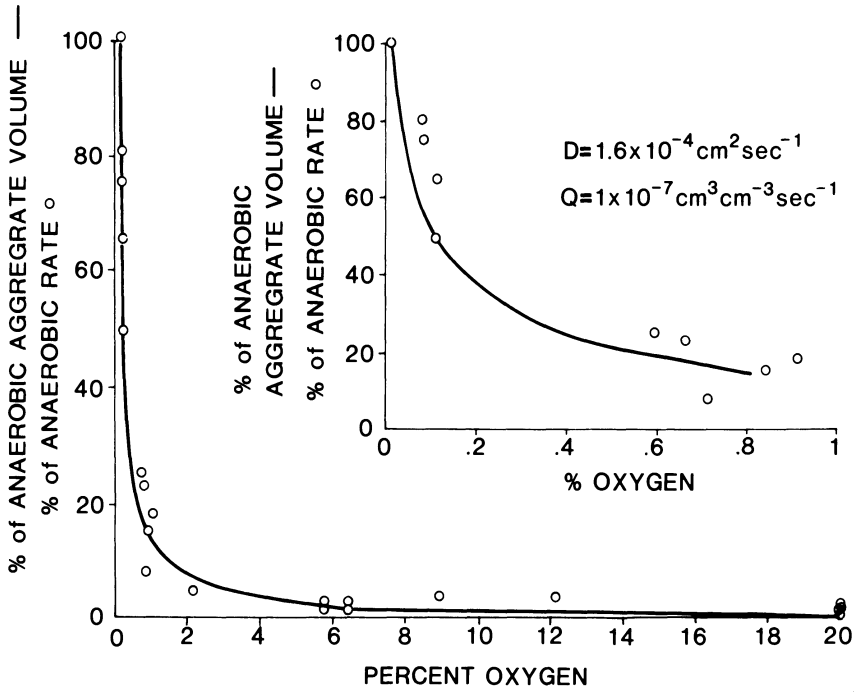

Fig. 3. Denitrification rates in a clay loam soil core at different pore oxygen concentrations (circles). Denitrification rates are expressed as a percentage of each core's anaerobic rate. The curve is percent of aggregate volume which was anaerobic as calculated by Smith's model of anaerobic volume. The inset shows an expanded view at low O_2 concentrations. Also shown are the diffusion coefficient of O_2 (D) and the O_2 consumption rate (Q) used to generate the curves. From Parkin and Tiedje[14].

of oxygen concentration suggests that the physical conditions imposed by the soil result in similar effects on the two measurements.

Fig. 3 also illustrates the strong inhibitory role of oxygen in restricting the activity of the denitrifying enzymes existing in soils. At 20% oxygen usually less than 5% of the potential activity was expressed. The denitrification rate increased as the oxygen concentration was decreased to below 2% where the rate increased dramatically. In nature, pore space oxygen rarely falls to this concentration in non-water saturated soils. Hence it seems unlikely that much of the potential denitrifying activity in soil is ever expressed.

We have used the acetylene core method to measure denitrification rates in the field and to study the effect of moisture on denitrification. These are detailed in the papers of Parkin *et al.*[11,12] and Sextone *et al.*[17] from this laboratory. As expected, denitrification often dramatically increased following rainfall or irrigation. In the two sites studied, 38 and 55% of the N loss occurred within 48 hours after moisture additions of greater than 1 cm[17]. This response to moisture shows

that it is important to accurately quantify denitrification N losses following rainfall and that sampling schedules must be determined by rainfall events if one hopes to achieve an accurate measure of N loss. We have also used geostatistical methods to determine whether there is a spatial component to the variability seen in denitrification rates. In the two sites analyzed so far we have found a spatial component sufficiently often to be encouraged that these techniques can be beneficially used in field studies of denitrification[13]. We have used punctual kriging to provide a contour map of denitrification rates for the study area and block kriging to provide improved estimates for denitrification losses over the block.

We have followed lines of British soil scientists – J. A. Currie, D. J. Greenwood, and K. A. Smith – interested in anaerobic microsites by extending their investigations to measurement of oxygen profiles within soil aggregates using a Clark-type, oxygen microelectrode. Revsbech and Ward[16] developed this electrode for work in hot springs and modified it to have thicker glass walls to be sturdy enough to insert into soil aggregates. Such electrodes have a 1 to 3 μm diameter membrane measuring tip and a response time of < 0.5 s, are stable for hours, and have a microscopic gold cathode so that the rate of oxygen consumption is insignificant. The sturdy electrodes used for soil aggregate had a shaft diameter of approximately 40 μm and were inserted at 0.1 mm increments with a micromanipulator. Details of the electrodes and results obtained with them are reported by Sexstone *et al.*[18]; some of the results are summarized here.

We studied aggregates from two Iowa silt loam soils, one in continuous cultivation for 50 years and the other from a nearby native prairie. The Iowa soil was chosen because of its low sand content since sand particles can damage or break the electrodes. The aggregates were moistened by placing them on water saturated filter paper for three days before study. Oxygen profiles within aggregates from the prairie and cultivated soil are illustrated in Figs. 4 and 5, respectively. The difference between these two profiles is greater than between populations of aggregates examined from the two sites, although the prairie aggregates had more root channels which caused more irregular profiles. These profiles illustrate that regular and irregular patterns can be measured by the electrode. As the electrode was withdrawn after insertion the oxygen readings during the withdrawal reproduced those measured on insertion which further confirm the accuracy of the method.

By inserting electrodes at different positions in a plane around the aggregate surface we were able to obtain contour maps of the

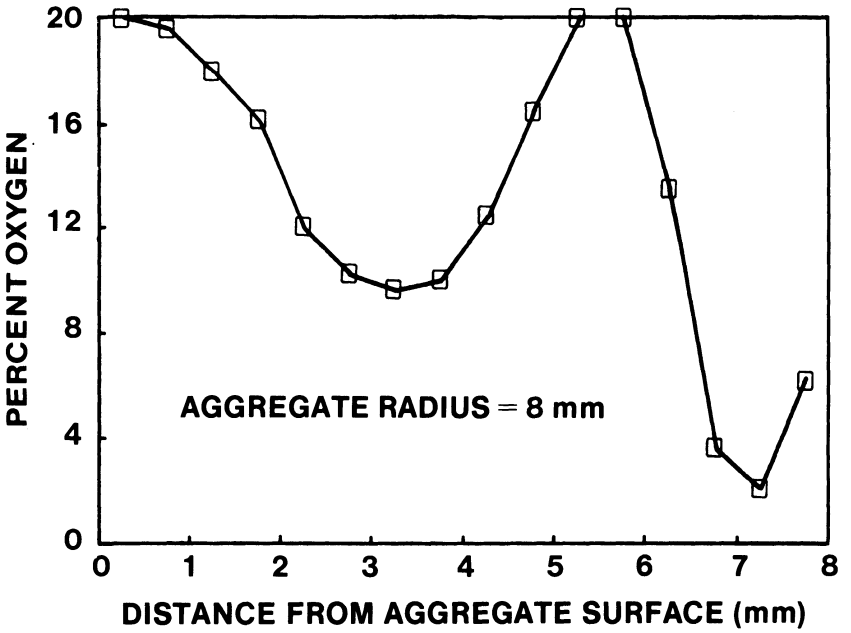

Fig. 4. Oxygen profiles within a silt loam aggregate from an Iowa native prairie soil. The discontinuity is due to an old root channel.

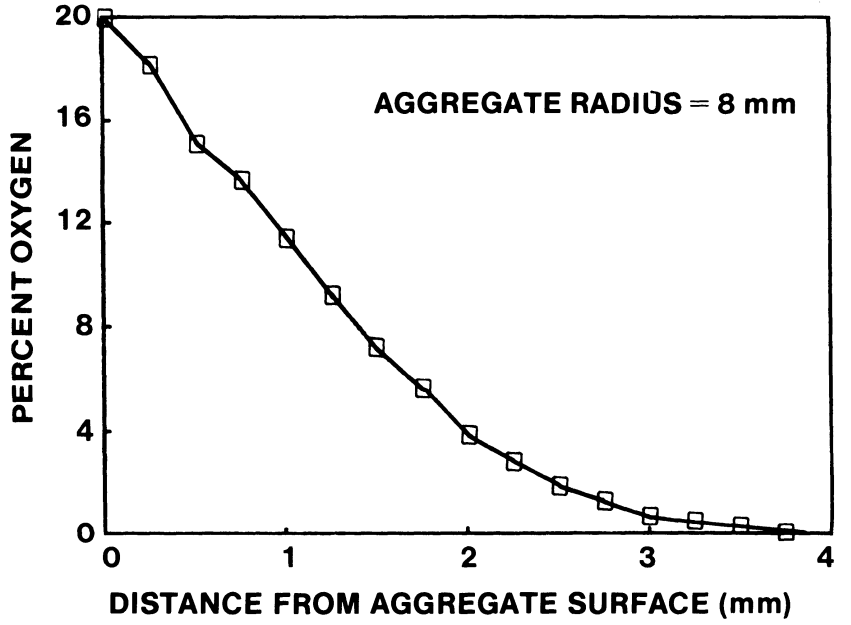

Fig. 5. Oxygen profile within a silt loam aggregate from an Iowa soil in continuous cultivation.

Table 2. Comparison of denitrification and anaerobic radii measured and calculated from cultivated and prairie soil aggregates. Taken from Sexstone *et al.*[18]

Aggregate	Denitrification rate ($ng - N \cdot g^{-1} \cdot day^{-1}$)	Aggregate radius (mm)	Anaerobic radius	
			Measured[a] (mm)	Calculated (mm)
CA4[c]	6.3	7.0	3.3	2.1
CA1	13.0	8.0	1.3	–[b]
CA2	9.2	12.0	5.5	5.7
CA3	2.3	13.0	5.4	7.2
PA1	0.03	9.0	0.0	–
PA2	1.7	18.0	14.0	5.6

[a] Mean radius measured on 4 to 10 profiles on each aggregate
[b] calculation indicates that no anaerobic radius should occur
[c] obtained from a cultivated silt loam (CA) or from adjacent uncultivated native prairie (PA)

oxygen profiles within aggregates[18]. These aggregates had radii of 6 to 9 mm; two of the three showed anaerobic regions near the center.

We also used the electrode to measure the intra-aggregate oxygen diffusion coefficient and, by gas chromatography, we have measured the aggregate respiration rate and denitrification rate. Smith[20] extended the models of radial diffusion to evaluate the extent of anaerobic zones within soil aggregates. Using his model and the measured oxygen diffusion coefficient, respiration rate, external oxygen concentration and aggregate radius we were able to calculate the predicted average anaerobic radii of the aggregates. As is shown in Table 2 there is good agreement between the measured and calculated anaerobic radii. The denitrification rates did not correlate well with the anaerobic volume, but too few aggregates have yet been measured to adequately assess this relationship. Denitrification rates were only measured in aggregates where a measureable anaerobic site was detected but not all aggregates with anaerobic sites denitrified.

Pathway to the N–N bond formed in denitrification

Despite the awareness of denitrification for a century it has never been resolved how the N–N bond is formed in denitrification. This is the key step in denitrification since it is the point of "no return" in which available N is lost to most of the biota. It is now well accepted that NO_2^- and N_2O are intermediates in denitrification, but the status of NO is less clear. NO is produced by all denitrifiers and thus must have some unique relationship to denitrification. Free NO does not seem to be an obligatory intermediate since in labeling experiments the N from NO_2^- reduction does not mix well with added NO[7]. However,

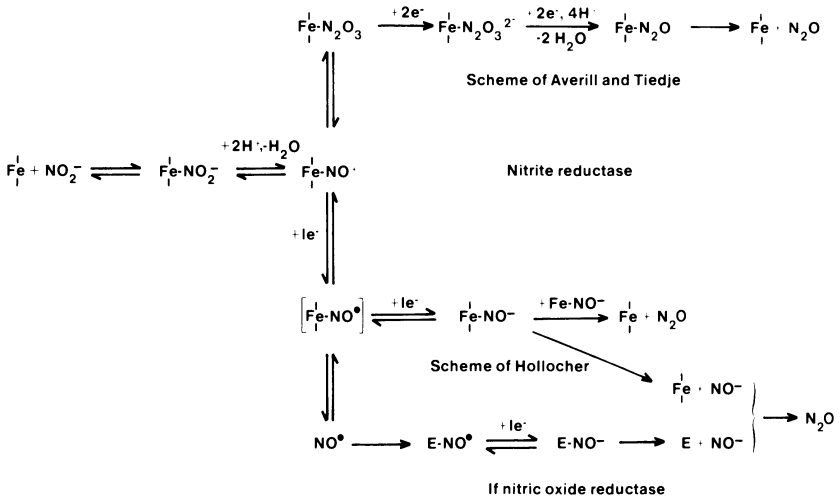

Fig. 6. Hypothesis of Averill and Tiedje[1] and Hollocher[7] for the formation of N–N bond in denitrification. The Fe represents the heme d binding site of nitrite reductase; E represents the NO binding site of nitric oxide reductase if such exists in the denitrification pathway.

it is also not clear whether NO occurs as a bound intermediate in the main denitrification pathway and whether there are one or two enzymes responsible for the conversion of NO_2^- to N_2O.

There are two nitrite reductases known to carry out denitrification. One type is a dimer with each identical monomer containing one heme c and one heme d. The heme d has been shown to be the binding site of nitrite. The second denitrifying reductase is less well studied but is known to contain two Cu atoms instead of the heme groups. Both enzymes seem to carry out the same reaction, and there is no reason to assume that there is any difference in the mechanism of N–N bond formation for each type of enzyme.

Averill and Tiedje[1] proposed a mechanism for the conversion of NO_2^- to N_2O that was based on chemically reasonable mechanisms and was consistent with all of the biological evidence (Fig. 6, upper portion)[1]. After the NO_2^- is bound to the heme, it is dehydrated to form a coordinated nitrosyl, a very reactive species. It is proposed that a second NO_2^- undergoes nucleophilic attack on the nitrosyl forming coordinated N_2O_3, the first species with N–N double bond. This species subsequently undergoes two successive two electron reductions through trioxodinitrate, $N_2O_3^{2-}$, and the dehydration to form N_2O. This scheme preserves the two electron per step reduction prevalent in biological reactions and avoids the reactive intermediate products caused by one electron reductions. In this pathway NO is

not an intermediate but it is expected to occur as a side product from the reaction nitrosyl.

A second pathway to N_2O has been proposed by Hollocher[7] which differs in that the reductive steps occur before N–N bond formation (Fig. 6, lower portion). The first step in this pathway are the same as proposed by Averill and Tiedje, but Hollocher now has strong evidence for them. He used a nitronyl trap to show production of the nitrosyl species and showed ^{18}O label exchange between nitrite and water as predicted from the reversible dehydration reaction[5]. After the nitrosyl species he proposes two one electron reductions through coordinated NO to form nitroxyl, NO^-. The nitroxyl dimerizes to form N_2O. In his proposal NO reductase (if one exists) could also contribute to the NO conversion to N_2O through a nitroxyl intermediate. Evidence is not yet conclusive for which of these two mechanisms is responsible for N_2O production. Knowledge of the mechanism could be useful in devising specific inhibitors of denitrification that would not affect other biological processes.

Anaerobic metabolism of organic compounds, especially dechlorination reactions.

Besides denitrification, fermentation is also of practical significance in soils. At the Letcombe Laboratory by Penn and Lynch[15] and associates have shown that acetate produced from decomposition of straw interferes with the establishment of cereal crops; an excellent example of problems that can be caused by fermentation. We have examined the anaerobic metabolism of manufactured chemicals from the perspective that there may be novel reactions carried out by anaerobes that may be useful in pollutant destruction. Most work on the environmental fate of pesticides and xenobiotic compounds has focused on aerobic metabolism. Although there are probably more aerobic biotransformations possible, this does not mean that there are no conversions of potential use. The most interesting reaction that we found is the reductive dehalogenation of the aromatic ring[21]. This is illustrated in Fig. 7 for some of the compounds that we have examined[4,21,23]. In this reaction the aryl chlorine is replaced by a proton. In anaerobic metabolism of chlorinated aromatic compounds the Cl is usually removed after ring cleavage; the latter is often blocked by Cl on the ring. Thus the anaerobic dechlorination represents a mechanism by which the Cl can be removed before ring cleavage thereby making the compound more susceptible to further aerobic or anaerobic degradation.

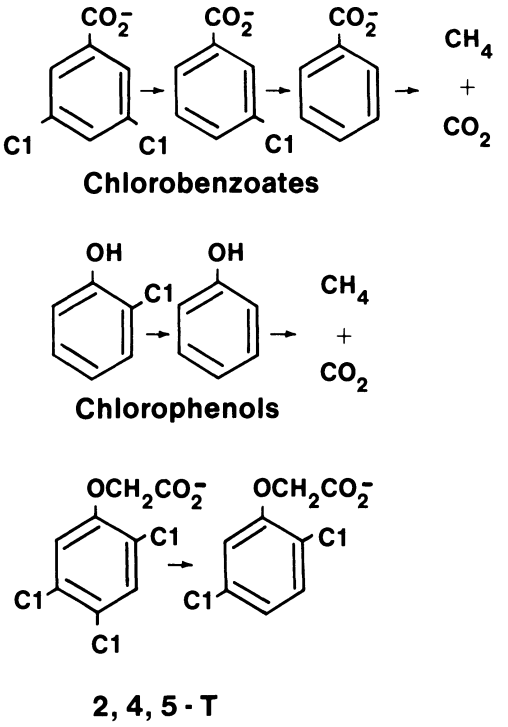

Fig. 7. Examples reductive dechlorination reactions that are carried by anaerobic methanogenic consortia.

We have been unable to enrich a methanogenic consortium from anaerobic sewage sludge using 3-chlorobenzoate as the sole C source[22]. This consortium dehalogenates Cl from the meta position of benzoate and Br from any position[21] and removes the para halogen from 2,4,5-T[23]. This consortium consists of members which dehalogenate the aromatic ring, oxidize benzoate, oxidize butyrate, and produce methane. We have now isolated the dehalogenating organism and find it to be a new, unusual organism both in morphology and physiology. The unique morphological feature is a "skirt" which surrounds the cell.

This unusual organism serves to illustrate the diversity that is probably yet to be discovered among anaerobes. This comment is also supported by the diversity now being recognized among the sulfate-reducing and methane-forming bacteria. Perhaps there is also more diversity of anaerobes in soils than is now recognized. If so, some of these organisms may have potential value. The dehalogenation reactions that we have examined so far have been studied in anaerobic sewage sludge and in lake sediments. Whether they exist in soil is not known, but it would not be surprising since the microbiota of soil

and sediments is not greatly different and anaerobic sludges are now added to many soils.

In summary, soils vary widely in extent and duration of anaerobiosis. Processes carried out by facultative anaerobes, particularly denitrification and fermentation, are the most prevalent and important anaerobic processes in soils. In well drained soils and soils from drier climates, anaerobiosis is of little importance. But in more humid climates or for poorly drained soils, anaerobiosis is of significance. With the new techniques now available a better understanding of the soil anaerobic environment is possible as is a better understanding of the biochemistry, physiology, and diversity of anaerobic organisms.

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Denitrification in field soils

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Key words Acetylene Arable crops Cultivation Denitrification Drainage Gas Chromatography Grassland Inorganic N fertilizer Irrigation Nitrogen Nitrous oxide

Summary Recent denitrification research is reviewed to answer questions a) how much N is lost from the soil as N_2 and N_2O and b) how do agronomic practices affect this loss? The methods used to quantify denitrification are also discussed. Gaseous losses of inorganic N range between the equivalent of 0 to 20 per cent of the fertilizer N applied to arable soils and 0–7 per cent on grassland soils. Losses are greater on undrained land and also after using direct drilling to establish arable crops.

Appendix 1 summarizes reported measurements of gaseous N losses.

Introduction

Denitrification brings the soil N cycle full circle as N is returned to the atmosphere. In taking stock of recent research we need to ask how much N is lost from soil and how do agronomic practices affect that loss? Estimates of such losses are not absolute as the amount is influenced by the interaction of a number of processes that affect the amount of nitrate in the soil. Typically about half the N applied as fertilizer is utilised in creating harvestable dry matter[15]; the remainder is temporarily retained in roots, immobilized into soil organic matter, leached or lost to the atmosphere. The vigour of the crop can affect these processes and hence alter the amount of nitrate that might be denitrified. Unfavourable growing conditions or major changes in management of the crop/soil system can greatly influence the eventual fate of applied N.

Nearly three decades ago Allison[1], reviewing lysimeter studies, suggested that gaseous denitrification losses of fertilizer N might be about 15 per cent. In British agriculture these emissions, if substantiated, would represent a loss of about 210,000 t of inorganic fertilizer N per year, costing about £66 m (1981 data). Since Allison published his review much research effort has concentrated on direct measurement of these losses and on determining the conditions permitting denitrification; this paper reviews results of recent research (1976–83) with particular reference to agricultural soils in the field.

During the period of this review Tiedje[70] sounded a note echoed in most recent reviews of denitrification that denitrification is the least understood sector of the N cycle in soils. Emphasis was laid

on $N_2:N_2O$ ratios, atmospheric N_2O, production and use of N_2O in soil, annual rates of N loss, physical processes at anoxic zone boundaries and the acetylene blockage technique. Payne[53] considered that denitrification is on a par with N fixation and photosynthesis as a process of great agronomic importance.

Denitrification

During denitrification NO_3^- serves as an electron acceptor for micro-organisms (usually facultatively anaerobic bacteria) with the result that the gases NO, N_2O and N_2 can be produced, mostly the latter two.

The population of soil micro-organisms varies from field to field, from soil to soil and from climatic zone to climatic zone, with about one-third of the soil bacteria being capable of denitrification. Pseudomonads are amongst the most common but several other kinds of micro-organisms can also produce nitrous oxide *e.g.* nitrifiers and NO_3 reducers.

Advances in methodology: 1976–1983

Estimates of N loss by difference methods

Indirect estimates of denitrification losses can be made by comparing inputs and outputs of N to a soil/crop system contained in lysimeters, especially when ^{15}N- labelled fertilizers are used. Although there is an extensive literature on such experiments, many results are of questionable value due to large experimental errors, or because the soil filling the lysimeter were not collected and preserved in their natural, field condition. The monolith lysimeters installed at Letcombe Laboratory were specifically designed to measure N balances, and to determine N_2O emissions directly[73]. However, measurements of N_2 loss could not be made as the acetylene inhibition technique was not available at the beginning of these experiments (1972–1976). In one experiment[20] losses of 12–18 per cent of applied ^{15}N labelled NO_3 were found, the discrepancy in the N balance being due to denitrification. The budget deficit method of assessing denitrification loss when carefully done is at least as good as more direct methods and should not be thought obsolete. Rolston *et al.*[58] attributed discrepancies between estimates from budget deficit and from direct measurements of fluxes using ^{15}N to under estimation by the direct method. This possibility should always be borne in mind when using one of the direct methods for estimating losses.

Estimates using isotopic tracers

This attractive method offers the potential of marking atoms of N

and observing them at different points along the denitrification pathway. This potential has been exploited using of the short-lived radioactive isotope, ^{13}N, by the research group at Michigan State University[71]. The use of the heavy isotope, ^{15}N, has continued as has the argument over the usefulness of variations in natural ^{15}N abundance[37]. ^{15}N is particularly good for tracing the fate of fertilizer-N but another approach is needed for measuring denitrification losses because labelling at high isotope enrichments is difficult and expensive.

Estimates from soil profile gas distribution

Large concentrations of N_2O in the soil atmosphere were first measured by i.r. spectroscopy of 1 litre samples[2]. Subsequently improved sampling techniques have been developed *e.g.* Dowdell *et al.*[18]. Estimates of N_2O emission have been attempted from its concentration gradient and diffusion coefficient in the soil atmosphere[10,59,67]. So far the method is not reliable because heterogeneity in the air-filled pore space results in large variability in concentrations and rates of transport of the gas. Nevertheless it has great potential for development, especially when microprocessors are used for repeated calculations.

Estimates by nitrous oxide emission

Many workers have measured N_2O emission by using sealed chambers covering a small area of soil[6,13,19,32,65,66]. From increases in N_2O concentration within the chamber over short periods (2–10 mins), rates of emission can be calculated. Several studies have been made of chamber design and effectiveness. Proponents can be grouped into those favouring static or dynamic atmosphere enclosures. The simpler static systems[6,23,31,33,49] appear to be as good as dynamic systems employing gas flow[13,66]. The other major advance has been in gas chromatographic systems for the analysis of N_2O using ultrasonic[4] and electron capture detectors[30,38,51,74].

N_2O is usually assumed to be lost from soil only by gaseous escape to the atmosphere. However, during winter it can also leave the soil dissolved in drainage water in amounts (0.25–4.4 kg N ha^{-1}) comparable to the gas phase emissions during the same period[16,17].

Estimates from laboratory measurement of potential denitrification

The simple aim of measuring the denitrification capacity of a field soil is complicated by the response of the denitrifiers to environmental changes. For example, one may take a sample of soil and subject it to conditions optimal for denitrification – anaerobiosis, warmth,

high moisture content, NO_3 supply. Denitrification proceeds first at a rate reflecting the initial status of the denitrifiers but rapidly attains a faster rate as the denitrifiers flourish. The latter is commonly regarded as the true potential but any pretreatment of the soil can have a marked effect on the result[43]. Are optimal conditions ever found in the field and if so for how long do they typically persist? In British agriculture warm, wet conditions with sufficient available nitrate in the soil may occur in the autumn (September–November) and again in the spring after fertilizer top-dressing (April–May) when surface soil may be saturated with water for one to seven days. This suggests that potential rates of denitrification should be measured over a similar period[9]. Even then such measurements are but a useful yardstick for comparison with *in situ* measurements and do not substitute for them. Limmer and Steele[44] successfully used an eight hour incubation to reflect seasonal changes.

Estimates using respiratory inhibitors

Direct field estimation of N_2 emission from soil has been difficult in the past because of the large concentration of N_2 in the atmosphere. Use of isotopes ^{13}N and ^{15}N (Smith *et al.*[68] and Tiedje *et al.*[71] is helpful only in limited circumstances. However acetylene inhibits the bacterial reduction of N_2O to N_2[22,77] resulting in the accumulation of readily measured N_2O. Field measurements of N_2O emissions after injecting acetylene into soil have been made in California[66] and laboratory evaluations of the method demonstrated stoichiometric recovery of N_2O in the presence of 0.1–1 per cent of acetylene[40,65,68,77]. Denitrification and overall soil respiration rates were apparently unaffected by acetylene but the inhibition remained effective for only 4–7 days in one trial with a silty clay loam soil[76]. In a field trial[9] after the initial peak caused by fertilizer and irrigation treatments, N_2O emission became steady at 0.05 kg N ha^{-1} day^{-1}, which was 10 times that from non-acetylene treated soil and 100 times the flux before N fertilizers were applied. Contrary to results from their laboratory experiments, the inhibitory effect of acetylene in the field lasted for 10–12 days, with the N_2O flux from treated soil decreasing almost simultaneously with the disappearance of acetylene from the soil.

Advances in knowledge: 1976–1983

The mole fraction of nitrous oxide (N_2:N_2O ratios)

Denitrification rate depends on temperature, being near zero around 0°C and maximum at about 40°C, then declining to zero at biocidal

temperatures. In agronomic practice, the range from sub-zero to 30°C is of most interest. McKenney *et al.*[48] have modelled this range using an Arrhenius equation: within it there is an important change-over in the dominant gaseous product from N_2O to N_2. At 7°C the two were in balance from a silt loam soil but at 25°C production of N_2 was much greater[39]. The denitrification rate in a sand loam doubled as temperature increased from 15°C to 25°C[14]. In two soils NO_3 was not denitrified at 1°C and was only slowly lost at 7°C[34].

Rainfall or irrigation stimulates denitrification by saturating the soil and limiting the oxygen supply; 20–30 mm rain may be needed to initiate denitrification in a dry soil[12]. Once anaerobic conditions were established N_2 was the dominant product from loam and sandy loam incubated at room temperature. After 3 hours N_2O production increased to half the gas loss but by 30 hours reduction to N_2 was again dominant. The denitrification rate was maximum between 7–12 h[25].

The supply of nitrate to denitrifying organisms in the soil can affect losses[55]. High concentrations of nitrate inhibit the reduction of N_2O to N_2[4] and so does nitrite[24,29], but it was less inhibiting than nitrate in another sand and in a clay in a separate experiment. So the situation is not clear: low concentrations of NO_3 ($5\,\mu g\ N\,g^{-1}$) stimulate the reduction of N_2O. Addition of N fertilizer affects the ratio of $N_2O:N_2$ in denitrification products; of the total gaseous losses N_2 amounted to only 30 per cent from soil with added fertilizer, but 75 per cent from unfertilized soil[9].

The available C supply can limit denitrification losses when NO_3 is plentiful but there have been few recent reports of the effects of soil C levels. In one study a pasture soil supplied more C for denitrification than an arable soil with a similar total C content[41]. CO_2 evolution is closely related to denitrification rates in laboratory studies[45]. Water-soluble C was closely correlated with denitrification over a range $5–130\,mg\,l^{-1}$ in topsoils[5] and $10–15\,mg\,l^{-1}$ in an actively denitrifying subsoil[28]. Addition of organic C to soil can slow down denitrification by increasing the immobilization of N in microbial cells[75].

Annual losses of nitrogen

Grassland The more intensively grassland is fertilized or grazed the greater the losses of N_2O and N_2 gas. Measurements of N_2O losses from grassland have been made in North America[52,60], Australia[13], the United Kingdom[61,73] and Germany[46]. Measurements of N_2 losses from grassland are rare[9,61].

NO is also released in measurable amounts from pasture soils, as

reported from Australia[27], but more quantitative measurements are needed.

From a four-year-old perennial ryegrass sward on a sandy loam soil denitrification losses were negligible[15]. The recovery of applied ^{15}N fertilizer in harvested herbage, water, soil and roots amounted to 98 per cent; the remaining 2 per cent being attributable to experimental error. On this soil, annual N_2O emissions did not exceed 0.04 kg N ha^{-1} (Dowdell and Webster[19]), but on clay and silt loam soils losses were greater[73]. Emission rates were consistently greater from clay soil with peak daily rates reaching 0.012 kg N ha^{-1} day^{-1} in autumn. During summer months, peak daily rates often reached 0.25 kg N ha^{-1} but lasted only for 2–5 days. Peaks were observed particularly following N fertilizer applications and significant irrigation or rainfall events.

Colbourn *et al.*[9] and Ryden[61] have both measured N_2 emissions from grassland using acetylene. Total gaseous losses from a Denchworth clay soil[9] were 2.1 kg N ha^{-1} over a period of 21 days following the addition of 210 kg N ha^{-1} as ammonium nitrate; three-quarters of the loss being N_2. Similar rates of N_2 emission were measured at another grassland site[61]. Annual denitrification losses (March 1980–February 1981) were calculated as 11 and 29 kg N ha^{-1} from plots receiving 250 and 500 kg N ha^{-1} respectively.

Arable land Several major studies of denitrification losses from arable land have been reported in the period 1976–1983 particularly from North America. Rolston and Broadbent[63] reported a comprehensive series of field measurements for the US Environmental Protection Agency. Emissions of N_2O were the chief interest but the use of ^{15}N labelled fertilizers also enabled measurements of N_2 emissions. Ryden *et al.*[64,65,66] reported a series of field measurements of N_2O flux, latterly using acetylene as a blocking agent to estimate N_2 losses. Mosier and Hutchinson[50] reported N_2O emissions from cropped land.

In Germany, Conrad and Seiler[11] reported N_2O losses from fertilizer N and Lippold and Forster[46] measured denitrification potential using ^{15}N to label both nitrate and ammonium in fertilizer. In the UK, a long series of measurements were made on cultivated land primarily of N_2 flux but latterly using acetylene blocking to estimate N_2 losses[6,9].

The accumulated evidence indicates that large quantitites of fertilizer N can be denitrified in arable soils, often between 10–30% of that applied. However, frequently the measured losses have been

small and not agronomically significant; also estimation of denitrification in the field is often made very difficult by the unpredictability of the weather[35].

Drainage Soil water content is one of the major factors influencing denitrification. In simple terms, the wetter the soil the greater the N loss. In one case a moderately well-drained soil contained about 200 kg of inorganic N ha^{-1} whereas an adjacent poorly-drained soil had very little[28]. Supporting evidence suggested that denitrification was largely responsible for the difference.

Although drainage should reduce denitrification losses, by removing water from the soil to give better aeration, it could have the opposite effect. Slightly better aeration in an inherently wet soil will stimulate microbial activity, increase the rate of turnover of N and increase the potential for denitrification particularly in zones of the drained soil that remain anaerobic or become anaerobic for a time. A recent evaluation of the acetylene inhibition technique[62] for grassland soils has underlined the need to adapt the technique accordingly to the wetness of the soil.

The installation of a mole drainage system in an arable clay soil changed the total gaseous losses and also the N_2O:N_2 ratio (Colbourn and Harper (in prep.)). In December gaseous loss from drained soil (0.043 kg N ha^{-1} d^{-1}) was all as N_2O but on undrained soil it was mostly N_2 (0.065 kg N ha^{-1} d^{-1}). Estimated maximum annual gaseous losses ranged between 16 and 50 kg N ha^{-1} from the drained plots and 24–110 kg N ha^{-1} from the undrained plots.

Drainage may reduce denitrification losses but it transfers more dissolved inorganic N from the cultivated soil into water courses, ditches and rivers[15]: as mentioned previously drainage water can hold measurable quantities of N_2O. NO_3 in drainage water is subject to denitrification at any stage of its journey in field drains and within stream courses: over one-half of the NO_3 load may be destroyed quite rapidly[69].

Irrigation Irrigation of arable and horticultural crops is common practice in many countries and often large amounts of N fertilizer are used. One study in the United States suggested that extra irrigation tended to diminish the denitrification losses from manured land[26] but the evidence was largely circumstantial, as the soil redox measurements showed that denitrification had exhausted the supply of NO_3 in the less irrigated soil at 80 cm depth. More irrigation gave rise to more NO_3 by mineralization and the total denitrification losses could

have been larger. Ryden and Lund[63] calculated N_2O loss from the number of days when the soil water suction was less than 300 mbars (about 15% soil moisture content) as 20–40 kg N ha^{-1} yr^{-1}. Notably, N_2O flux declined sharply at suction 50 mbar (24% N_2O content), presumably because of reduction of N_2O to N_2.

Irrigation of a warm grassland soil will result in denitrification of substantial amounts of fertilizer N. In 20 days, at 23°C, a ryegrass sward lost 4.3 kg N ha^{-1} as N_2O and 24 kg N ha^{-1} as N_2 from 300 kg N ha^{-1} applied as potassium nitrate. The calculated losses of N_2O reported by Ryden[61] and Webster and Dowdell[73] from rainwetted or irrigated grassland were 0.2 kg N ha^{-1} d^{-1} after fertilizer applications were perhaps 1–2 kg N ha^{-1} lost from 63 kg N ha^{-1} ammonium nitrate[61] or 100 kg N ha^{-1} calcium nitrate fertilizer[73]. Ryden[61] reported an equal amount lost as N_2 in these conditions with daytime temperatures 17–24°C. Rainfall (120 mm) increased N_2O loss from 0.001 kg N ha^{-1} d^{-1} to 0.01 kg N ha^{-1} d^{-1} on native prairie grassland that had received fertilizer N 40 days previously[52]. Denitrification from slurried animal excreta[21] applied to a gley soil under pasture in July 1981 occurred only after irrigation (53 mm) and persisted for a few days (cumulative loss 2 kg N ha^{-1}).

Denitrification potential is very high in paddy soils and urea based fertilizers must be applied carefully to minimize losses[72].

Cultivation method

On a Denchworth clay soil, emission from direct-drilled arable plots during the period November 1977 to June 1979 was nearly always greater than from ploughed plots, by factors up to 15-fold[6]. During the highest peak periods (late autumn and spring) rates of N_2O loss from direct drilled plots were 0.6–0.7 kg N ha^{-1} day^{-1}. A similar pattern of emission was observed on Lawford clay soil, but rates were only 10–20 per cent of those from Denchworth soil. On both soils the amounts of N lost annually were greater from direct-drilled treatments (1.5–8.6 kg N ha^{-1}) compared with ploughed soil (0.5–5.6 kg N ha^{-1}). In an eight day period between 31 May and 7 June 1979 after heavy rain (40 mm in 10 days previously) ploughed plots lost N_2O equivalent to 2.2 kg N ha^{-1} while direct-drilled plots lost 3.3 kg N ha^{-1}. Estimates of total loss from silt loam soil[56] and from clay soil (Colbourne, Harper, personal communication) were larger from direct-drilled than ploughed soil.

Fertilizer Although estimated losses of plant available N by N_2O emission are only rarely of agricultural significance[15], large amounts

will be lost when fertilizer N is applied to a wet, warm soil, or when the soil subsequently becomes wet.

Early lysimeter work with a sandy loam supporting a ryegrass sward indicated no significant gaseous losses from 70 kg N ha^{-1} applications as calcium nitrate[19]. Losses ($N_2O + N_2$) of 3–31 kg N ha^{-1} from 100 kg N ha^{-1} applications as ammonium nitrate have now been reported[47] but only 1–2 kg N ha^{-1} as N_2O from 70 kg N ha^{-1} as calcium nitrate[73]. Urea added to prairie grassland trebled the loss of N_2O, 0.5 kg N ha^{-1} being lost from 85 kg N ha^{-1} (Mosier *et al.*[53]). In the UK large daily rates (0.2 kg N ha^{-1}) were observed only when ammonium nitrate fertilizer (250 and 500 kg N ha^{-1} y^{-1}) was applied to cut grassland during periods of frequent rain and when soil temperatures were 5 to 8°C[61]. These denitrification peaks persisted for only 2–3 weeks and in the spring of 1981 amounted to 15 per cent of that applied; denitrification was negligible when ammonium sulphate was used. During the winter of 1981–82 continuous denitrification occurred only in lysimeters treated with ammonium nitrate (100 kg N ha^{-1}) and amounted to a loss of about 40 kg N ha^{-1} (Eggington and Smith[21]).

Additions of ammonium nitrate in June 1980 resulted in an increased rate of denitrification to 0.14–0.3 kg N ha^{-1} d^{-1} with 55–78 per cent of this loss being in the form of N_2O[9,10]. Further studies on undrained, direct drilled plots, using ^{15}N labelled NO_3 (Colbourn *et al.*[10]) showed that denitrification during May accounted for 45 kg N ha^{-1} with 90 per cent of the gas emission as N_2, equivalent to 45 per cent of that added at the beginning of the month. Denitrification losses from spring barley grown on a shallow soil overlying chalk for four years, amounted to 12–18 per cent of the applied ^{15}N labelled NO_3 (Dowdell and Webster[20]).

Fallow land Few studies have been made on fallow land although this is traditionally one way of maintaining soil fertility.

Modelling denitrification

Enough isolated facts are now known for a conceptual, mechanistic model of denitrification in arable soils to be derived in the hope of predicting or estimating losses with reasonable accuracy.

There are several approaches to the modelling of denitrification in soils. The microbiological enzyme kinetics and population dynamics may be modelled by reference to standard formulae of growth and function[7]. Alternatively, the soil can be treated as a physical system with solid, aqueous and gas phases, the rate limiting step being diffusion of gasses[42,67]. Another possibility is to model the environmental

processes, mostly those related to rainfall and temperature[48] along with soil nitrate concentration. Waste water management problems have given rise to models of nitrate rich waters moving through soil profiles involving nitrate concentrations, time, soil depth and temperature factors[3,8,36].

Conclusions

1. Direct estimates of gaseous losses of inorganic N by denitrification range between the equivalent of 0–20 per cent of the fertilizer N applied to arable soils and 0–7 per cent on grassland soils (see Table 1).

2. More development work is required on the acetylene method before even recent estimates of total denitrification losses can be considered fully reliable.

3. More research is needed to develop strategies for minimizing denitrification losses. Particularly lacking is an understanding of the population dynamics of denitrifying micro-organisms and how they respond to changes in soil management practice especially drainage and incorporation of crop residues.

4. Perhaps there is need for a reappraisal of the merits of wholly ammonium-N, wholly nitrate-N and mixtures of the two fertilizer forms in relation to denitrification losses.

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Appendix 1. Summary of reported measurements of gaseous N losses from soil/plant systems

Author	Peak daily rate kg N ha^{-1} d^{-1}	Estimated Annual losses kg N ha^{-1}	Remarks
In soil atmosphere			
Colbourn and Harper *In prep.*	0.6–1.6	45	Based on calculation from $^{15}N_2$ concentration 100 kg ha^{-1} NO_3-N, arable, 13°C.
Indirect estimates by lysimeter studies			
Dowdell *et al.*[17]	–	zero	^{15}N balance of grass on sandy soil
Dowdell and Webster[20]	–	11–22	^{15}N balance of spring barley on chalk soil
Direct measurement of emissions			
Dowdell and Webster[19]	–	0.04	N_2O Grass on sandy soil
Webster, Dowdell[73]	0.012–0.25	4.8	N_2O Grass on clay and silt loam
Burford *et al.*[6]	0.02–0.7	0.9–9	N_2O winter wheat on clay loams
Ryden[61]	0.2	11.29	$N_2O + N_2$ grassland on clay soil
Colbourn *et al.*[9,10]	0.05–0.11	18–38	$N_2O + N_2$ grassland on clay soil, 210 kg N ha^{-1}, 12°C
Colbourn *et al.*[9,10]	0.01–0.07	7–13	$N_2O + N_2$ winter wheat clay soil, 70 kg N ha^{-1}, 19°C
Colbourn and Harper, *In prep.*	0.04–0.3	16–110	$N_2O + N_2$ winter wheat on drained and undrained clay soil, 140 kg N ha^{-1}
Egginton and Smith[21]	0.25	40	$N_2O + N_2$ on slurry and fertilizer treated pasture
Ryden *et al.*[65]	0.7–1.9	51	14% N_2O, summer arable with 335 kg ha^{-1} nitrate-N
Hutchinson and Mosier[32]	0.001–0.3	2	N_2O summer arable with 200 kg ha^{-1} NH_3-N
Ryden and Lund[63]	0.2–1.0	8	N_2O, 336 kg ha^{-1} NH_3-N
Rolston and Broadbent[57]	2–60	34–208	5–12% N_2O, 23°C, 300 kg N ha^{-1}
Rolston and Broadbent[57]	1–3	18–31	5–16% N_2O, 8°C, 300 kg N ha^{-1}
Losses in drainage			
Dowdell *et al.*[17]	–	0.25	N_2O dissolved in drain water
Dowdell[16]	–	1–4.4	N_2O dissolved in drain water

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Total denitrification and the ratio between N_2O and N_2 during the growth of spring barley

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Key words Barley Denitrification *Hordeum vulgare* N_2 N_2O

Summary Total denitrification ($N_2O + N_2$) and nitrous oxide emission were measured on intact soil cores using the acetylene inhibition technique.

Total denitrification from the depth 0–8 cm during the growth period from April to August was 7 kg N/ha from plots supplied with 30 kg N/ha and 19 kg N/ha from plots supplied with 120 kg N/ha. The amounts of precipitation, plant growth, and N application were found to affect the denitrification rate. These factors also affected the ratio $(N_2O + N_2)/N_2O$, which varied from 1.0 to 7.2. Plant growth and precipitation increased the proportion of N_2 produced, whereas a high nitrate content increased the proportion of N_2O.

Introduction

From an agricultural point of view denitrification is a very important process since considerable amounts of plant-available N may be lost by this process.

Estimates of denitrification losses have been made in several cases and by various methods[1,4,8,11,12]; and losses varying from a few to about 70 per cent of the applied N have been reported. One source of variation could be, that when total denitrification is estimated only from measurements of N_2O, a fixed overall ratio between N_2O and N_2 production is used, and since this ratio is known to vary depending upon factors such as carbon supply[12], soil treatment[9], plant growth[14], and nitrate content in soil[3], the estimate may be erroneous.

In the present investigation the acetylene inhibition technique[17] and measurements of N_2O emission have been combined to estimate the total denitrification, and to calculate the proportion of N_2O and N_2 produced during the growth period of spring barley. Effects of N application, plant growth, and precipitation were also studied.

Materials and methods

Experimental area

Samples were taken from a field plot experiment with continuous barley cultivation started in 1973. The plots are located on a sandy loam soil at Roskilde State Research Station on Plant Science, Sealand. Physical and chemical properties of the soil are described by Vinther[15].

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Two levels of N fertilization were selected for these studies, one below (30 kg N/ha/year) and one above (120 kg N/ha/year) the normal application to barley in this area (90 kg N/ha/year). The N was applied as calcium-ammonium-nitrate (50% ammonium and 50% nitrate) on April 21, and at the same time the barley (cultivar Zita) was sown. Phosphorus and potassium (400 kg/ha of a PK-fertilizer containing 19 kg P, 49 kg K, and 10 kg Mg) were applied in the autumn the year before. Total precipitation during the growth season was 331 mm and the mean temperatures in the months April, May, June, July, and August were 5.7, 12.5, 13.6, 15.4, and 15.4°C, respectively.

Sampling and treatment of samples

Samples for determination of N_2O production were taken to a depth of 8 cm as intact soil cores in steel tubes 35 mm in diameter. Cores were taken both with and without plants. Samples without plants were taken between two barley rows spaced approximately 10 cm apart. These samples therefore were not free from roots, but the density of roots in samples with plants was considerably higher than in samples without. The size of the incubation vessel and the sampling procedure made it necessary to decapitate the plants about 5 cm above the soil surface before sampling.

Samples for determination of nitrate in the soil by extraction with 2*M* KCl for 1 h were taken several times during the growth season.

Measurements of denitrification

All samples were taken as pairs with four replicates. From each pair one sample was incubated with acetylene and the other without acetylene, in order to calculate the ratio

$$N_2O \text{ produced with } C_2H_2/\text{without } C_2H_2.$$

If acetylene completely inhibits N_2O reduction, the calculated ratio is equal to

$$(N_2O + N_2)/N_2O.$$

Results from samples incubated with acetylene correspond to total denitrification. The loss of N in the form of NO was considered to be negligible.

Immediately after arriving at the laboratory the samples were incubated in glass containers (volume 390 ml) fitted with a rubber septum. Samples to be incubated with acetylene received 10% acetylene after removing 10% of the air with a gastight syringe. To obtain activities as near as possible to field activities the incubated samples were placed under outdoor conditions.

Measurements of N_2O, both in samples with and without acetylene, were performed the day after sampling and again 24 h later and the N_2O production was calculated by difference.

Analytical procedure

N_2O was measured by a gas-chromatograph with an electron capture detector as described by Vinther *et al.*[16].

Results

Fluctuations of denitrification ($N_2O + N_2$), NO_3-N in soil and precipitation during the period from April to August are shown in Fig. 1. Before fertilization denitrification was low, probably due to low temperatures and to lack of substrate (nitrate). After application of N denitrification increased only slightly until mid-May while the soil was very dry due to low precipitation. In the last week of May the highest activity occured, coinciding with heavy rainfall, and thereafter the denitrification rate decreased to a constant low level not

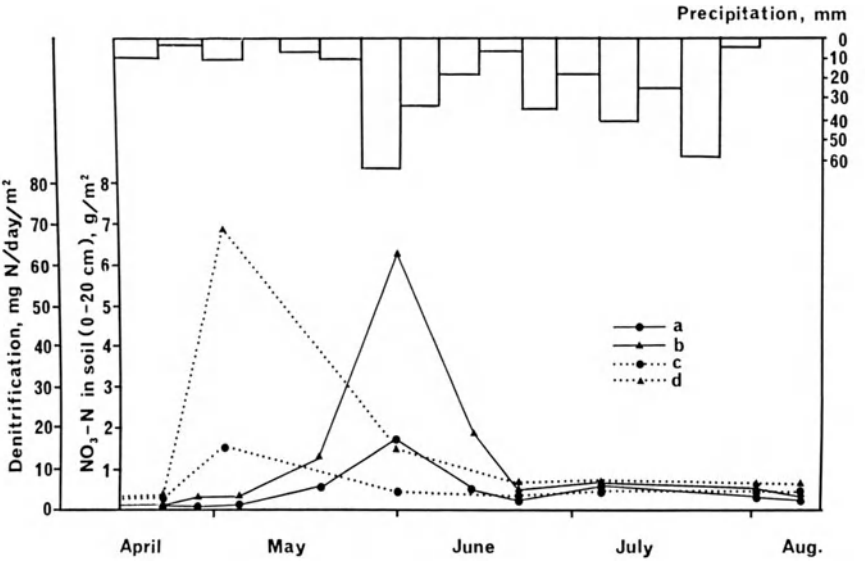

Fig. 1. Denitrification, NO_3-N content in soil and precipitation during the growth period of barley.
a = Denitrification from plots applied with 30 kg N/ha
b = Denitrification from plots applied with 120 kg N/ha
c = NO_3-N content in plots applied with 30 kg N/ha
d = NO_3-N content in plots applied with 120 kg N/ha.

affected by fluctuations of precipitation. In this six week period precipitation and therefore also soil moisture were relatively high but most of the nitrate was used by the plants and low activities of denitrification were measured.

The denitrification losses shown in Fig. 1 amount to 7 kg N/ha from plots supplied with 30 kg N/ha and to 19 kg N/ha from plots supplied with 120 kg N/ha. These are mean values of samples with and without plants.

In a previous investigation[2] no significant difference was found between the denitrifying activity in the depth 0–5 cm and 5–20 cm, and activities in deeper layers were found to be insignificant. In the present investigation denitrification was measured in the depth 0–8 cm, and therefore the total denitrification from plots supplied with 30 kg N/ha and 120 kg N/ha can be estimated to be approximately 15 kg N/ha and 50 kg N/ha respectively.

In Fig. 2 the effect of plant growth on the denitrification shows that when supplied with 30 kg N/ha the presence of plants on an average increases the activity by 2.5 times, whereas when supplied with 120 kg N/ha the increase is 5 times. In the samples without plants there was no difference between the denitrification from the two N levels, but if

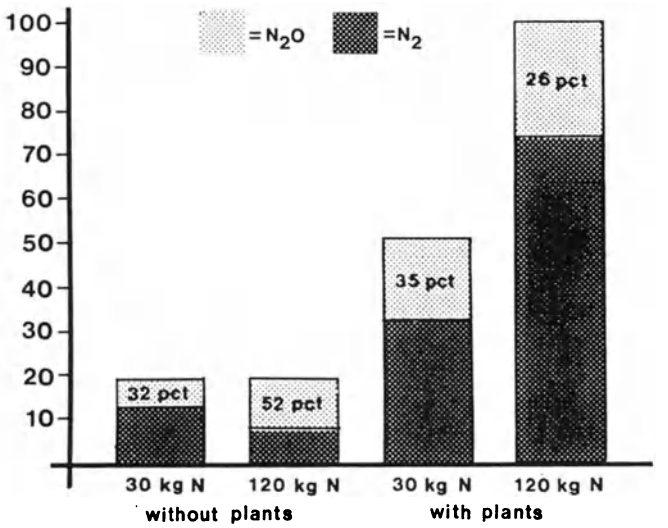

Fig. 2. Relative denitrification and ratio between N and N_2O per cent.

plants were present the denitrification was about twice as high in plots supplied with 120 kg N/ha as in plots with 30 kg N/ha. This indicates that supply of nitrate does not increase the denitrification rate unless other factors like oxygen tension and carbon supply are optimized also.

Fig. 2 also shows the mean ratios between N_2O and N_2 in the four treatments; the ratios at each sampling during the growth period are shown in Table 1. In the absence of plants, increasing the amount of fertilizer decreases the ratio $(N_2O + N_2)/N_2O$, which means that the proportion of N_2O is increased. In plots supplied with 30 kg N/ha, 32% of the N loss was N_2O, whereas the corresponding percentage in plots supplied with 120 kg N/ha was 52% (Fig. 2). In the presence of plants the opposite effect was observed.

Within each of the four treatments the ratio between N_2O and N_2 was relatively constant throughout the growth period, except 31 days after germination (Table 1), when a drastic increase in the amount of N_2 occurred. This increase coincided with a period of heavy rainfall (Fig. 1).

Both plant growth and precipitation (soil moisture) have a positive effect on denitrification by consuming oxygen and restricting oxygen diffusion; but also the ratio between N_2O and N_2 seems to be affected by the availability of oxygen. At low oxygen tension the proportion of N_2 is relatively larger than at higher oxygen tensions. Application of N fertilizer has an opposite effect on the ratio between N_2O and N_2.

Table 1. Effect of plant growth and nitrogen application on the ratio $(N_2O + N_2)/N_2O$ during the growth cycle of barley

Days after germination	Without plants		With plants	
	30 kg N/ha	120 kg N/ha	30 kg N/ha	120 kg N/ha
4	1.2	1.1	3.3	1.9
18	3.0	1.3	3.0	3.9
31	6.5	4.9	4.2	7.2
45	4.0	2.0	2.4	3.4
52	2.3	1.4	2.5	4.3
67	2.0	1.0	–	2.8
94	2.8	1.4	2.1	3.0
Mean	3.1	1.9	2.9	3.8

Discussion

From the available literature it is evident that losses of N due to denitrification vary considerably depending upon various factors such as soil type, irrigation or rainfall, cropping pattern, and fertilization management. Burford and Stefanson[6] found a maximum N_2O flux corresponding to 0.28 kg N/ha/day from a sandy loam soil fertilized with 100 kg N/ha. Since they could not measure the N_2 produced from the denitrification they could not account for all gaseous losses from the soil. A calculation of the maximum N_2O flux in the present investigation gives 0.25 kg N/ha/day, which is in good agreement with the findings of Burford and Stefanson.

Total denitrification has been estimated in a number of investigations[1,5,11,12,13] and losses of gaseous N corresponding to values from 0 to 70% of the fertilizer N applied have been reported. In the present investigation the total loss of gaseous N corresponded to 50% and 42% from plots supplied with 30 kg N/ha and 120 kg N/ha respectively.

In this investigation the factors which chiefly influence denitrification and cause variations in the rate of denitrification appear to be: (1) soil moisture, which at high levels stimulates denitrification by restricting O_2 diffusion, (2) plant growth, which may stimulate denitrification by providing carbon substrates and consuming soil O_2, and (3) soil nitrate level. These factors have also been shown to affect the proportions of N_2O and N_2 produced. Both increasing soil moisture and plant growth reduce oxygen availability in the soil and increase the proportion of N_2 produced, whereas high levels of nitrate increases the proportion of N_2O produced during the denitrification. Similar trends have been reported by Dowdell[7], Stefanson[14] and Blackmer and Bremner[3].

The mean ratios between N_2O and N_2 found in the present investigation are similar in magnitude to those stated by Lensi and Chalamet[10], but the variation from 1.0 to 7.2 indicates that it is unrealistic to use overall ratios for calculating total denitrification from N_2O measurements or to estimate N_2O losses from estimates of total denitrification.

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Nitrite: a key compound in N loss processes under acid conditions?

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Key words Chemo-denitrification Iron oxyhydroxide Nitrate Nitric oxide Nitrite Nitrogen Nitrogen dioxide Reduced conditions Soil

Summary Nitrite is very important in N transformation processes because it is an intermediate product in the aerobic nitrification as well as in the anaerobic denitrification process. Under soil conditions whereby aerobic and anaerobic zones are close to each other, the mobile nitrite can be a link between both N transformation processes. Because of its low stability in acid conditions, nitrite can be a key compound in N loss processes.

The results are presented in three sets of incubation experiments using soil + added nitrite before and after oxidation of organic matter; soil + added nitrite and various iron oxide minerals; nitrite solutions without soil but with added ferrous iron.

It was found that under acid conditions, soil organic matter as well as the soil mineral phase have a stimulating effect on the nitrite decomposition. Conditions favouring the solubility of Fe(III)-compounds and promoting the formation of Fe^{2+} increase the nitrite decomposition, even under slightly acid conditions. Of the gaseous decomposition products, only trace amounts of NO_2 occur while NO is the major component. Conditions whereby NO and NO_2 cannot escape from the medium promote production of some nitrite.

Introduction

Nitrite is rarely found in nature[3]. Only occasionally does nitrite accumulate in soils and aquatic systems, depending on soil characteristics and agricultural practices such as ammonium fertilization, soil or water pH, organic matter content, temperature and moisture content and soil-fertilizer geometry[4,8,11,12,16,18,19,34]. All these factors induce nitrite formation if they promote alkaline conditions. Indeed, several experiments have shown that nitrite is rapidly decomposed in acidic or mildly acidic soils[12,14,26,29,37]. The decomposition of nitrite is controlled not only by self-decomposition in acid conditions but also by interaction with soil constituents. Both metallic cations and organic matter have been claimed as being partly responsible for nitrite decomposition[10,26,27,40]. Furthermore, because of its injurious effect on plants, accumulation of nitrite is undesirable[5,16].

In the nitrogen transformation processes, nitrite is very important because it is an intermediate product in aerobic nitrification as well as in the anaerobic denitrification process. Under soil conditions whereby

aerobic and anaerobic zones are close to each other, the mobile nitrite can be a link between both nitrogen transformation processes.

The aim of the authors is to increase understanding of nitrite stability and its decomposition products under acid conditions. To this end three sets of experiments were performed as follows:

Material and methods

Soil-nitrite suspensions

Different amounts (100 and 1000 mg) of a silty loam soil (pH 6.15 and % C = 1.15) were added to nitrite solutions containing 40 μg NO_2^-–N buffered at pH 3.8, 4.2, 4.6 and 5.0 with sodium acetate-acetic acid (0.2 *M*) in 100 ml closed flasks. No attempt was made to sterilize the added soil, as it was found that nitrite stability is independent of sterile or non-sterile conditions[6,9]. Preliminary experiments showed that the acetate ions did not have any influence on the nitrite stability. After different periods of time, separate duplicate samples were analysed for nitrite, using the Griess–Ilosway method[7]. The experiment was repeated with the same soil after the organic matter was destroyed by H_2O_2 treatment. All incubations were carried out at 25°C.

Soil–iron compounds–nitrite suspensions

In 500 ml closed conical flasks, 250 mg of powdered haematite, goethite, lepidocrocite and amorphous iron oxyhydroxide were mixed with 125 g of the same silty loam and brought into suspension by adding 375 ml H_2O. These flasks were incubated anaerobically at 25°C for one year. As such, depending on Eh and pH of the soil suspension and on the solubility product of the solid Fe(III)-compounds, different amounts of Fe^{2+} were formed in the suspensions. Then, 40 μg/g NO_2^-–N on a soil basis were added and followed as a function of time.

Nitrite decompositjon products in the presence of ferrous iron

Nitrite solutions (40 and 80 μg/g NO_2^-–N) were incubated in 100 ml flasks in the presence of 600 μg/g Fe^{2+}. A series of incubations were carried out under a continuous He stream, while another series were incubated under closed conditions, the air being replaced by He. The pH values of the experiments were 4 and 6. For the open incubation, the gases produced were removed by the He stream during 18 hours and collected by selective absorption solutions. For the closed incubations, the He stream was only started after 18 hours. The He streams containing NO and NO_2, were passed successively through a triethanolamine solution and through the $KMnO_4$ solution. Formed NO_2 was absorbed by a triethanolamine solution, containing guaiacol and Na-metabisulfite[25]. Several authors[2,21,22] have shown that this solution quantitatively absorbs NO_2 leaving NO unchanged. The alkaline $KMnO_4$ solution absorbs NO + NO_2[15,28]. N_2O and N_2 are not absorbed by these solutions. After 18 h, the residual NO_2^-–N as well as the formed NO_3^-–N were determined. The absorbed NO_2 in the triethanolamine solution was measured by a nitrite determination, using the Griess–Ilosway method. The amount of NO + NO_2 in the $KMnO_4$ solution was determined by a nitrite analysis through distillation.

Results and discussion

Soil-nitrite suspensions

Fig. 1 shows log [NO_2^-–N] in the different treatments as a function of the incubation time. The highly significant linear relationship between both parameters strongly suggests that the nitrite decomposition

Table 1. Reaction rate constants and half-life times (T1/2) of 40 μg/g NO_2^-–N at different pH values and amounts of soil

	pH							
	3.8		4.2		4.6		5.0	
mg soil	100	1000	100	1000	100	1000	100	1000
k_1 (day^{-1})	0.21	0.37	0.12	0.28	0.03	0.05	0.03	0.03
T1/2 (day)	3.3	1.9	5.6	2.5	26.9	13.7	51.0	27.6

follows first order or pseudo first order kinetics, as was also found by Laudelout *et al.*[20] It can be seen that decreasing the pH as well as addition of soil stimulate nitrite decomposition. The increased decomposition rate at lower pH values can be explained by taking into consideration that nitrous acid rather than the nitrite ion is involved in the decomposition. The dissociation constant of HNO_2 ($pK_a = 3.14$) indicates that the pH of the suspension should be low to very low in order to have significant self-decomposition. It should be stated here that the pH at the reaction site in soils can be lower than the pH of the bulk soil[17,23], shifting the equilibrium between HNO_2 and NO_2^- towards HNO_2. Also theoretical considerations concerning nitrite decomposition indicate that above pH 5.47 nitrite self-decomposition is excluded[35].

The first order reaction rate constants and the half-life times of NO_2^-–N in the different treatments are given in Table 1. Self-decomposition and interaction with soil constituents have a cumulative effect. Increasing the amount of soil from 100 to 1000 mg under the experimental conditions decreased the half-life of nitrite by about half. The contribution of the organic fraction of this soil can be seen from Table 2, in which the decrease of the nitrite concentration is compared in the presence and absence of organic matter. It is clear that the absence of organic matter decreases the nitrite decomposition rate by

Table 2. Decomposition of NO_2^-–N (μg/g) at different pH values, in the presence of 1000 mg of soil, treated with (+) or without (—) H_2O_2

Incubation time	pH							
	3.8		4.2		4.6		5.0	
(days)	+	—	+	—	+	—	+	—
0	39.0	38.5	38.8	39.2	39.4	39.8	39.7	40.6
2	27.7	24.4	31.4	29.1	37.5	36.4	38.9	39.1
4	19.1	16.1	25.3	21.0	35.8	33.1	38.8	37.4
9	5.2	1.5	10.8	5.9	32.1	25.1	36.9	32.0
12	3.0	0.3	7.3	1.5	26.9	21.5	34.9	28.6
Mean difference	2.6		3.4		3.2		2.3	

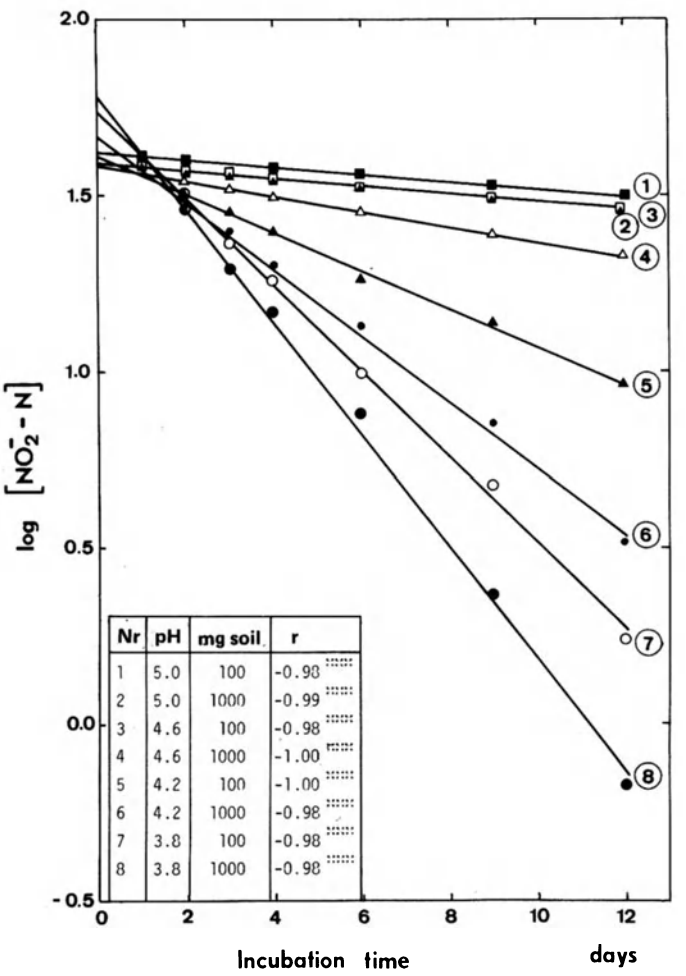

Fig. 1. Log [NO_2^--N] as a function of time in the presence of 100 and 1000 mg of soil at different pH values.

about 5 to 10% of the initial concentration. This positive effect of soil organic matter was also found by Clark *et al.*[14]; Reuss and Smith[29]; Smith and Clark[31] and Stevenson and Swaby[32].

Soil–iron compounds–nitrite suspensions

Depending on pH, Eh and the solubility of the iron oxyhydroxide products, a certain amount of Fe^{2+} is formed in the soil. The decomposition of NO_2^-–N added to these soil-Fe redox systems is given in Fig. 2: the pH and Eh of the respective systems are given. It can be seen that even in slightly acid conditions the nitrite decomposition rate is

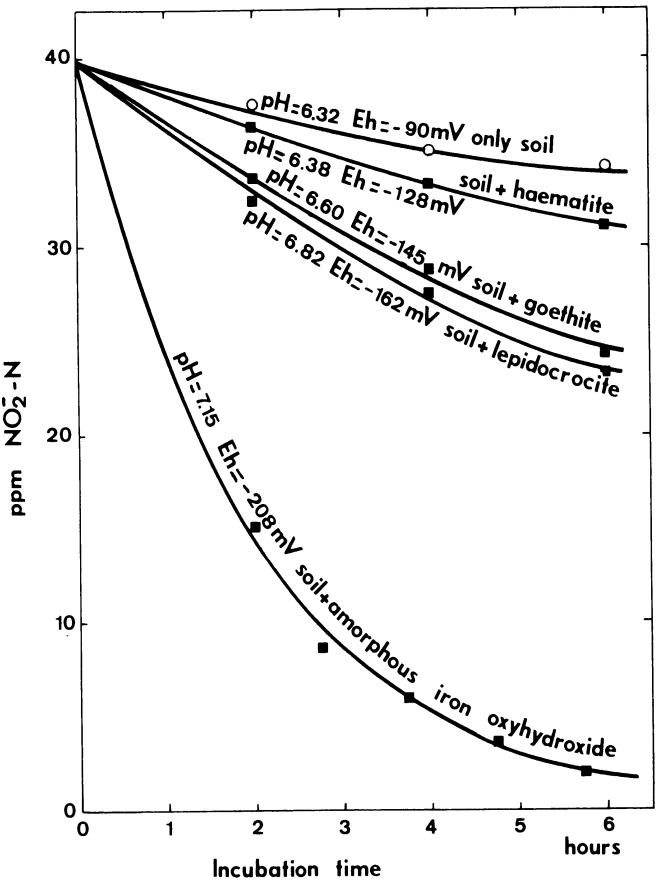

Fig. 2. Effects of iron oxides on decomposition of nitrite in soil suspensions.

variously increased by the treatments with crystalline iron oxides and tremendously so by amorphous oxyhydroxide. There is an important difference between the crystalline materials (goethite, haematite, lepidocrocite) and the amorphous material. Indeed, the amorphous material is about 10^4 times more soluble than crystalline material, bringing more Fe^{2+} into solution. The increasing influence of increasing amounts of Fe^{2+} was also demonstrated by Chalamet[10]; Van Cleemput and Baert[36] and Wullstein[39]. Their experiments, however, did not cover the influence of different solid Fe(III)-compounds. A question might arise about the simultaneous occurrence of Fe^{2+} and nitrite. It is, however, suggested that this is possible at the boundary of aerobic and anaerobic zones in the soil.

Nitrite decomposition products in the presence of ferrous iron

The results of the nitrite decomposition study, given in Table 3, show that the highest amount of residual nitrite was found under

Table 3. Nitrite decomposition products

pH	Added NO_2^--N (μg/g)	Residual NO_2^--N after 18 h μg/g	%	NO_2-N μg/g	%	NO-N μg/g	%	NO_3^--N μg/g	%	Total (μg/g)	% Recovery
						OPEN					
4	40	0.7	1.8	0.19	0.5	39.17	97.9	0.0	0.0	40.1	100.2
	80	1.3	1.6	0.16	0.2	75.33	94.2	0.0	0.0	76.8	96.0
6	40	39.6	99.0	0.00	0.0	0.53	1.3	0.0	0.0	40.1	100.3
	80	70.5	88.0	0.01	0.0	8.06	10.1	0.0	0.0	78.6	98.2
						CLOSED					
4	40	9.5	23.8	0.57	1.4	19.30	48.3	2.5	6.3	31.9	73.4
	80	26.5	33.1	1.00	1.3	33.82	42.3	5.2	6.5	66.5	76.7
6	40	39.0	97.5	0.01	0.0	0.85	2.1	0.0	0.0	39.9	99.7
	80	78.0	97.5	0.01	0.0	1.57	2.0	0.0	0.0	79.6	99.5

open conditions at pH 6. The amount of NO_2 was very small, while the amount of NO was large. At pH 4, a very fast decomposition rate was noted. Indeed, besides self-decomposition, also Fe^{2+} speeds up the decomposition rate. After 18 h of incubation, almost all NO_2^-–N had disappeared. Nitric oxide was the most important decomposition product, while NO_2 generally accounted for less than 1%. The total recovery was around 100%. Although gas-chromatographic analysis (thermal conductivity detector) did not show any N_2O, there might have been a small N_2O or N_2 production. Under closed conditions, at pH 6, almost all nitrite stayed unchanged. Only a trace of NO_2 and a small amount of NO was found. The total recovery was nearly 100%. At pH 4, the total recovery was considerably lower. A small amount of NO_2 and NO_3^-–N and much NO was detected. It is supposed that under closed conditions the formed NO was further reduced to N_2O and N_2. The formation of these products is reported in the literature[28,30,31,38].

According to different authors[1,24,28,33] the following nitrite decomposition reactions are proposed:

$$3\,HNO_2 \longrightarrow HNO_3 + 2NO + H_2O \qquad (1)$$

$$2\,HNO_2 \longrightarrow NO + NO_2 + H_2O \qquad (2)$$

Also it appears that nitrate may be formed indirectly from NO_2 according to reaction (3)

$$2NO_2 + H_2O \longrightarrow HNO_2 + HNO_3 \qquad (3)$$

If the N-oxide gases escape from the medium the reaction equilibrium shifts to the right, so increasing nitrite loss. The small amount of nitrate formed only under closed anaerobic conditions, indicates that reactions (1) and (2) occur simultaneously with a dominance of reaction (2).

It can be concluded that, whatever nitrite is formed in a soil under acid conditions, soil organic matter as well as the soil mineral phase have a stimulating effect on the nitrite decomposition. Conditions favouring the solubility of Fe(III)-compounds and promoting the formation of Fe^{2+} increase the nitrite decomposition even under slightly acid conditions. Of the gaseous decomposition products, only trace amounts of NO_2 occur, while NO is the major component. Conditions whereby NO and NO_2 cannot escape from the medium favour formation of some nitrate.

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Section 5

Dynamics and models of organic carbon, nitrogen and phosphorus transformations in cultivated soils

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The biological transformation of P in soil

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Key words Immobilisation Microbial P Mineralisation Organic P P transformations Soil P cycle

Summary Organic forms of soil phosphorus (P_o) are an important source of available P for plants following mineralisation. The rates and pathways of P through soil organic matter are, however, poorly understood when compared to physico-chemical aspects of the P cycle. The essential role of soil microorganisms as a labile reservoir of P, confirmed experimentally and in modelling studies, has recently led to the development of methods for measuring their P content. Incorporation in a new P fractionation scheme of these measurements with estimates of P_i and P_o fractions that vary in the extent of their availability to plants has enabled the dynamics of short-term soil P transformations to be investigated in relation to long-term changes observed in the field.

Different types of soil P compounds that mineralise at different rates can now be measured directly in extracts by ^{31}P-nuclear magnetic resonance. Orthophosphate diesters, including phospholipids and nucleic acids, are the most readily mineralised group of these compounds. However, mineralisation rates rather than the amounts of types of P_o in soil ultimately control P availability to plants. These rates are influenced by a number of soil and site factors, as a sensitive new technique using [^{32}P] RNA has recently shown.

These recent developments reflect a more holistic approach to investigation of the soil P cycle than in the past, which should lead to improved fertilizer management practices.

Introduction

The enormous numbers of microorganisms that inhabit soil mostly live in a state of near starvation, having a survival strategy that depends on the maintenance of ATP levels and an adenylate energy charge – a measure of the stored metabolic energy – similar to that of actively growing microorganisms *in vitro*[6].

Phosphorus has a key role in this strategy, participating in most significant metabolic pathways, as well as being a structural component of many biochemicals including nucleic acids, co-enzymes, phosphoproteins and phospholipids. Consequently, both plants and soil organisms actively compete for P from the meagre levels of orthophosphate (P_i) (*ca.* 1.5 μM) maintained in soil solution by the chemical processes of precipitation-solubilisation and adsorption-desorption. Compared to these processes, the rates and pathways of P through soil organic matter are poorly understood, and involve extremely complex

interrelationships between detrital processing, mineralisation of organically-bound P and P uptake mechanisms. The biological transformations of P in soil most likely to influence the P nutrition of plants are assessed here in the light of recent advances in the investigation of the soil P cycle (for recent review, see Stewart and McKercher[32]).

Evidence for the availability of P_o to plants

Several types of evidence collectively indicate an important role for soil P_o in plant nutrition, in temperate as well as tropical soils.

The depletion of organic matter induced by cultivation is well known and in the few cases where the fate of the P in organic matter has been measured, the P_o fraction frequently accounts for most or all of the loss recorded[36]. For example, up to one half of the average P removed from some English arable soils in cereal crops came from organic forms by mineralisation[26]. Further evidence is provided from grassland soils, where net accumulation of fertilizer P as P_o continued to about the same maximum level, irrespective of P application rate or grazing pressure, when the mineralisation and immobilisation rates are in balance[40]. Liming can hasten the establishment of this balance, presumably by creating a more favourable environment for increased biological activity, and increasing the solubility of stable P_o forms like inositol polyphosphates. Precipitation on lime particles of the P_i released by mineralisation can, however, delay its availability to plants[19].

The high available P status of New Zealand pasture soils in spring, when pasture growth rates are high, is thought[30] to be caused by the release of P from organic matter by mineralisation. Periods of rapid growth in a cool temperature grassland soil coincided with increases in labile ($NaHCO_3$ – extractable) P_o, highest microbial activity, and lowest levels of P associated with the litter[15].

Finally, simulation modelling studies of both natural grassland[9] and grazed pasture[3] also indicate that mineralisation of P_o supplies a significant proportion of the plant's total P requirement; such studies have stimulated further research on soil P by revealing gaps in knowledge about the mineralisation process, the role of phosphatases in this process, and the influence of the microbial biomass on the transformations of P in soil between organic and inorganic forms.

Most of these examples, however, give little indication of the mechanisms involved. How much of the P_i mineralised from organic matter in a growing season actually reaches the plant, and how much becomes immobilised in microbial cells or chemisorbed on clay surfaces? The

answer to this question will emerge as the mechanisms involved become better understood. A general consideration of the soil P cycle provides a framework for discussion of these mechanisms.

The soil P cycle

The part of the P cycle of most immediate importance to plants, and soil organisms, is summarised by the following equilibria:

$$\text{soil solution P} \underset{\text{very fast}}{\rightleftharpoons} \text{liable } P_i \underset{\text{very slow}}{\rightleftharpoons} \text{non-labile P}$$

Rapid dissolution of the labile P_i fraction recharges the soil solution P against uptake by growing plants and the soil biota.

In undisturbed ecosystems, where the labile P_i fraction is small and the P cycle tightly closed, most of the P entering plants is supplied from the slow recycling of plant residue P through microbial processes in the soil. This is why Cole *et al.*[9] chose a natural ecosystem in a semiarid grassland for constructing their simulation model of the soil P cycle. Transfer rates between pools in the model indicated that labile P_o made a large contribution to the soil solution by mineralisation.

In agricultural systems, the P cycle is more open because agricultural products are removed, and losses often occur from surface runoff and erosion. Consequently, fertilizer P is added to compensate for these losses, as well as to raise plant production above natural levels, making the contribution of the soil cycle more difficult to assess. In both natural and agricultural systems, the incorporation of plant, animal and microbial residues (above and below ground) into the soil provides the energy needed to sustain nutrient cycles, as well as ensuring that the P they contain re-enters the P cycle. Grazing animals accelerate this re-entry[27], although much of the P released from residues is immobilised in decomposer organisms, reducing the net amount released into soil solution by leaching. Although P_o mineralisation depends primarily on the activity of soil microorganisms, invertebrates, especially earthworms, have an important regulatory function in this process. Surface casting earthworms, for example, can increase the short-term availability of P in plant residues by 2- or 3-fold through the release of (mainly) P_i in plant material by physical disruption; this is especially important in soils of low P status[25]. The net functional contribution of invertebrates to cycling, however, depends largely on their ability to stimulate microbial activity[22]. This is very difficult to measure in field soils and so the rhizosphere has been simulated in the laboratory. Sterilised soil and added consumer and decomposer organisms (microcosms) have been used in various combinations to investigate

the role of the microfauna (*e.g.* amoeba, nematodes) in the soil P cycle[10].

Soil microorganisms comprise an important source and sink for nutrient elements like P, as well as being the main agents for transforming these elements. In an unmanured English arable soil, for example, a P flux through the microbial biomass of 4.6 kg P ha^{-1} y^{-1} was calculated, which compared with about 5 kg P ha^{-1} y^{-1} removed in grain-plus-straw[23]. Comparative efficiencies of P_i uptake from soil by microorganisms and plant roots, although difficult to measure, have been estimated from in vitro experiments[2] and simulation models[9] of the P cycle, and both indicate that microorganisms, especially bacteria, have been efficient uptake mechanisms.

Competition for the small amount of P in the soil solution is most intense in the rhizosphere where substances from root exudates, sloughed-off root cells, tissues and mucigels sustain a larger and more active microbial population than in the bulk soil. Although rhizosphere microorganisms benefit the growth of plants, the mechanisms are poorly understood. These effects in the short term probably result more from growth hormones produced by microorganisms than from increased availability of P from non-labile sources[38]. Nevertheless, plants and microorganisms actively scavenge for P by several possible mechanisms. Plants, for example, can change the rhizosphere pH by organic acid production or net excretion of H^+ or HCO_3^- ions[14]. P_i can also be released from organic forms, mainly ester P, through the action of extracellular phosphohydrolases, that are produced in response to a need for P. Soil phosphatase activity has been used with mixed success as a simple index of P_o mineralisation[31]. The problems associated with this approach are formidable, firstly because the chemical nature of the P_o substrates in soil is still largely unknown, and secondly, the interrelationships between different enzyme pools are poorly understood.

In summary, the equilibrium concentration of P in the soil solution, and the capacity of the soil to maintain this concentration against uptake by plant roots and microorganisms, are governed by complex physico-chemical and biochemical mechanisms. McGill and Cole[24] used the term biochemical mineralisation to distinguish the latter mechanism, involving the release of ester-P and S by extracellular enzymes, from the mechanism for the mineralisation of C, N and C-bound P (biological mineralisation) where a need of soil organisms for energy is the driving force. These concepts can accommodate the well-known variability of C:N:S:P_o ratios and the greater variability of P_o than S in soil organic matter, and also provide a rationale for understanding the stability of P_o in different soils. The balance between net mineralisation

and immobilisation depends on the C:P ratio of the substrate actually being decomposed, rather than on the ratio for soil organic matter or plant residues in general. This critical C:P ratio was calculated recently, from microbial growth yield and C:P values, to fall in the range of 50 to 70[39].

Characterisation of these soluble forms of soil P_o, and of P_o in the solid phase have presented soil scientists with a formidable challenge, with most effort in the past having been made on the latter.

Forms of soil P and their characterisation

Chemical characterisation of soil P

Soil P occurs in a wide variety of different inorganic and organic forms. P_i is found mainly in combination with Al and Fe oxides and hydroxides, clay minerals, solid carbonates and soil organic matter. It also occurs in small quantities as pyrophosphates and polyphosphates, of mainly microbial orgin[28].

Soil P_o determination is still one of the outstanding problems of soil analytical chemistry, because P_o can be estimated only approximately by indirect ignition or extraction methods[1]. The chemical diversity of soil P_o, which occurs mainly as mono- and diesters (*i.e.* $(RO)PO_3H_2$ and $(RO)(R'O)PO_2H$), is chiefly due to the organic moieties R and R'. Inositol hexakis- and pentakisphosphates, primarily of plant origin, comprise up to 60% of soil P_o, with smaller amounts of phospholipids and nucleotides, and traces of sugar phosphates, phosphoproteins, glycerophosphate and phosphonates also present (for review, see Tate[35]). A large proportion of the remaining P_o is still chemically unidentified, but probably occurs as insoluble complexes with clay minerals and organic matter. Much of the P_o in soil extracts has a high molecular weight, and includes phosphorylated inositols; some macromolecules in these extracts may, however, be artefacts[12].

A new approach to the problem of characterising soil P_o involves the use of ^{31}P-nuclear magnetic resonance (nmr) to obtain both qualitative and quantitative[28] estimates of the various forms of P in alkaline extracts of soils. In one soil, myo-inositol hexakisphosphate was confirmed as the main orthophosphate monoester.

Despite the continuing progress being made towards identification of the different chemical forms of P_o in soil extracts, future prospects for the quantitative separation of unaltered P_o from soil are not very encouraging. The chemical complexity of soil P_o, the susceptibility of some P_o compounds to hydrolysis during extraction, strong sorption of

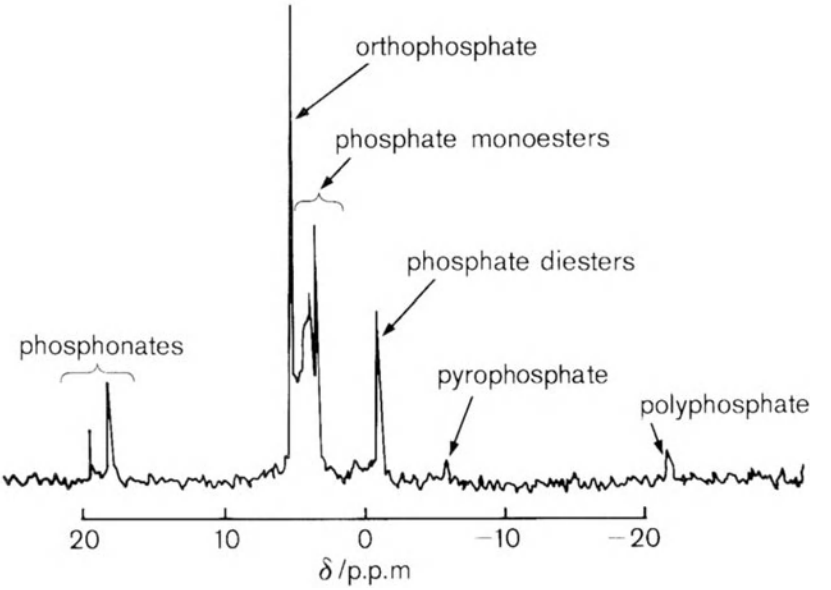

Fig. 1. ^{31}P-nmr spectrum of the alkali extract from an acid (pH 4.1) New Zealand high country soil (Typic Dystrochrept) showing the different forms of P (Newman and Tate[20]).

P_o by clays, and the formation of insoluble salt with metal cations, are all complicating factors.

Microbial P

The concept developed during the past decade[23] of the microbial biomass as a small but labile reservoir of nutrients in soil has stimulated considerable interest in the role of organic matter in plant nutrition. Several methods for its measurement in soil are in common use, and recently, two methods were described for measuring the P content of the microbial biomass[5,20]. Microbial P was previously estimated from the microbial biomass, using literature values for the P contents of laboratory cultured microorganisms. These P contents, however, can vary widely depending on the age of organisms, the growth conditions, and the P content of the growth medium. A reliable method for measuring soil microbial P must take account of the wide range of P compounds in soil, their diverse chemistry in microbial cells, and their extractability from soils differing widely in adsorption capacities. These criteria have largely been met in the two new methods. They are similar in principle, and based on the widely used $CHCl_3$-fumigation procedure for measuring soil microbial biomass. The P content of the microbial biomass is calculated from the difference in the amounts of P extracted

with 0.5 *M* $NaHCO_3$ (pH 8.5) from fumigated and unfumigated portions of soil. A factor (kp) provisionally set at 0.4, corrects for the fraction of this P extracted after fumigation. The validity of both methods depends to a large extent on how closely this kp applies to a native soil population, and this has still to be established. The two methods do differ in some important respects. Greater precision was attained in one method, for example, by the removal of labile P_i from air-dried, ground soil prior to extraction of the fumigated and unfumigated soils[20]. In the other method, the use of field-moist soils was preferred because air drying markedly reduced the P_i released by fumigation[5].

Differences in extractable P_i between fumigated and unfumigated soil were used by Brookes *et al.*[5] to indicate microbial P, employing a spike of P_i to correct for fixation during fumigation, and extraction of the P_i from lysed cells. In the other method, each soil was calibrated for the recovery of microbial P, because total P (P_T) rather than P_i was found to be a more reliable index of the P released from microbial cells. Despite these differences, the two methods provide an opportunity to define more clearly the role of microorganisms in the P cycle. Just how useful these methods will be on a wider range of soils than the near neutral to alkaline soils used to develop them is difficult to judge without further extensive testing. However, results from some acid New Zealand topsoils under high-producing pasture appear promising. The microbial P contents of 21 topsoils (0–75 mm; pH range 4.9–6.8), measured by the Brookes *et al.* method ranged from 20 to 88 $\mu g\, g^{-1}$ dry soil (mean 51 $\mu g\, g^{-1}$)[29]. These values are equivalent to 11–57 $kg\, ha^{-1}$, representing 0.5–11.7% of the P_T, which is similar to the range reported for some British soils (0.5–11.9%)[5].

Characterisation by chemical extractants

Research of P cycling in grassland soils has recently stimulated interest in methods for separating soil P_o fractions according to their activity in the P cycle, and led to the development of a fractionation scheme based on the use of different soil extractants[4]. Four P pools were identified in this scheme – labile P extracted with 0.5 *M* $NaHCO_3$, moderately labile P alkali-soluble P_i and P_o (obtained by extraction with acid), moderately stable (fulvic acid) P and highly stable (humic acid) P. Although the fractionation procedure was reproducible and indicated P movement between different fractions, further refinements were considered necessary[7].

A similar but more comprehensive scheme was proposed recently[21]. This allows the determination of recognised P_i fractions (labile,

secondary, occluded, and primary minerals), the separation of labile and stable forms of P_o, and the measurement of microbial P.

P transformations and microbial processes

Short-term changes in the P cycle caused by the addition of organic residues and P have recently been investigated in laboratory incubation experiments using these fractionation procedures. These experiments have revealed the transfer rates of P between P_i, P_o and microbial P that eventually lead to the changes in P distribution often observed in the field. For example, when C was added every 30 d as dried grass, or as cellulose (with and without added P), to a Chernozemic black soil, 39 and 22% respectively of the P in the grass and added with the cellulose were found in organic forms[7]. Microscopic observation of bacterial and fungal biomass indicated maxima 4–5 d after each addition. Depletion of soil P_i over the 9 month period of the experiment with the addition of cellulose alone suggested that eventually, mineralisation of P_o would be necessary as labile P_i became exhausted. This was confirmed in a subsequent experiment involving two soils with widely different available P_i status[8]. The efficiency of the mineralisation process was indicated by the similar amounts of added C remaining in both soils after the +C (57%), and +C,P (53%) treatments. Microorganisms took up soil solution P during the experiment, and microbial C:P ratios ranged from 12 for the soil with a large reserve of available P_i to 45 for the soil with limited reserves.

These laboratory investigations have been extended using the sequential extraction technique to elucidate the mechanisms involved in changes in P_i and P_o resulting from long-term cropping in a wheat-wheat-fallow rotation, compared to an adjacent permanent pasture[21]. Cropping reduced P_T by 29%, of which 74% was P_o and residual P. Of the P_T lost, 22% came from extractable P_o and 52% from stable forms, indicating that considerable microbially-mediated mineralisation had occurred. An explanation for these field results was sought from laboratory incubation experiments, again by adding C (as cellulose) with and without P to two soils with widely different P_i status. In the P_i-deficient soil, with a microbial C:P of 45, both C and P additions were needed to cause an increase in P_o; P_o mineralisation resulted from adding C alone. This result contrasted with the P_i-rich soil, where C additions with and without added P caused P_o to increase, mainly as P-rich microbial detritus (microbial C:P *ca.* 15).

An even clearer picture of the dynamics of the soil P cycle would be obtained if the fate of discrete P compounds in soil could be followed.

Table 1. Forms and amounts of P in alkali extracts of a permanent grassland soil and an adjacent bare fallow soil (20 y), calculated from total P (P_T) contents of extracts and relative peak areas in ^{31}P-nmr spectra

Form of P	Alkali-extractable P ($\mu g\,g^{-1}$ oven-dry soil)	
	Highfield grass	Highfield fallow
Inorganic orthophosphate	23	89
Orthophosphate monoesters	61	75
Orthophosphate diesters	53	7
Unknown	21	20
P_T†	158	191
P_i†	24	99
P_o†	134	92

† Measured by chemical analysis of extract.
Adapted from Hawkes *et al.*[18]

For example, a soil development sequence in New Zealand tussock grasslands was recently used to show that diester P, probably originating from microorganisms, comprised the most important source for available P_i, given soil and climatic conditions favouring mineralisation[34]. Mineralisation of diester P was also mainly responsible for the 41% decline in total P_o observed when an English grassland soil (pH 6.1) was ploughed and kept fallow for 20 y (Table 1)[18]. The phosphonate discovered in the moist acidic soils of the tussock grassland sequence was believed to have originated from the soil protozoan, *Tetrahymena pyriformis*.

Phosphonate P has subsequently been found in a lowland English soil; again, low soil pH (*ca.* 4.6), by restricting decomposition, was responsible for a wider variety of P_o forms than found in an adjacent permanent grassland with a higher pH of 6.1[18].

The ultimate limit on the availability of soil P_o to plants and microorganisms is the rate of P_i release by mineralisation, rather than the amounts of P_o present. Mineralisation rates of P_o have in general been estimated from incubation experiments, by monitoring changes of P_i and P_o with time. These methods are, however, rather imprecise. Problems arise from soil heterogeneity, the effects of strong extractants on labile P compounds, and the difficulty of accurately estimating recoveries of P_i and P_o from some soils. P transformations between different pools can be measured with considerably more precision by using isotopes, rather than net flows obtained by conventional procedures. This advantage has recently been incorporated in an incubation method that uses [^{32}P] RNA to estimate the mineralisation rates of labile P_o in soil[16]; (RNA is part of the labile P_o pool[4], comprising up to 50% of the P in microbial cells). In 50 English woodland soils, where P_o

mineralisation is the main factor limiting P availability, this new method showed that the variations in labile P_o mineralisation rates were mainly explained by soil pH and levels of extractable calcium[17]. The technique has considerable potential for further elucidation of the effects of soil and management factors on P transformation. It could also provide answers to questions about the relative uptake by plants and micro-organisms of the labile P_o during a growing season. ^{32}P-labelled bacteria have been used[33] to follow the availability of the label to growing plants, indicating that of the 20% that appeared after 26 d in the resin-extractable form, about 70% was taken up by the plant. Although the ^{32}P-labelled bacteria would probably have decomposed more rapidly than the native soil population, the experiment nevertheless demonstrates the availability of microbial P to plants and underlines the need to include plants in experiments designed to elucidate pathways in the soil P cycle.

The many factors that control the population dynamics and activities of soil microorganisms also control the balance between mineralisation and immobilisation of P in soil. They include temperature, moisture, aeration and the soil reaction (for reviews see Dalal[13], Tate[35]). Plants also influence the rates and pathways of P in soil actively[14], as well as through recycling of nutrients in plant residues[37]. The influence of these various factors on the dynamics of the soil P cycle ultimately control P availability to the plant.

The need to quantify P transformations in soil is illustrated in simple pragmatic models like Superchoice[11] that already provide objective advice to farmers on the use of fertilizer P. In this model, superphosphate requirements necessary to offset P losses resulting from grazing animals, and from transformations into slowly available soil forms, are estimated. These soil losses must, however, be estimated indirectly from field experiments and animal losses, because direct measurements are not possible. However, a better appreciation of the rates and pathways of P through the organic matter in soils, and of the interaction between the biological and physico-chemical processes controlling the P cycle should lead to the refinement of predictive models like Superchoice, with ensuing economic benefits to agriculture.

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Modelling C and N turnover through the microbial biomass in soil

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Key words Biomass turnover Carbon Experimental verification Models Nitrogen

Summary Many mathematical descriptions of C and N transformations in soils have been developed in the last decade, but only a few explicitly model the activity and mass of soil organisms. Great difficulties still exist in establishing basic parameters governing the kinetics of microbial turnover. The present state of the art is discussed briefly.

The model of Van Veen and Frissel on C transformations and related mineralization and immobilization of N has been developed further based on laboratory and field data obtained with different Australian soils. Firstly, case studies show the large effects of the frequency of drying and rewetting of soils on the decomposition of organic matter and on the turnover of biomass. Secondly, the more refined model embraces the concept that soils have characteristic capacities to preserve both organic matter and microorganisms. Preservation of microorganisms could result from protection against predation and/or from amelioration of harsh environmental conditions. Biomass formed in excess of a soil's preservation capacity is assumed to die at a relatively high rate. Furthermore, biomass and its immediate organic products of decay are considered to form mainly a closed system from which only small proportions of the products leak out as stabilized materials.

These concepts have been tested with data from laboratory experiments in which ^{14}C- and ^{15}N- labelled substrates and bacteria were added to a clay and sandy soil. Net mineralization of C and N (labelled and unlabelled) and changes in the total and labelled biomass as determined by the chloroform fumigation technique allowed for a thorough testing of these concepts in the manner in which they were included in the model. The fits between experimental observations and model outputs were very close. The model indicated that the contrasting metabolism of both C and N in a clay *versus* a sandy soil could largely be explained by differences in the capacities of the two soils to preserve microorganisms.

The ability of a simulation model to describe accurately not only short-term events, *e.g.* N cycling during one growing season, but also the same processes over, say a decade, is an important criterion in assessing its predictive power. In this paper some of the results will be discussed of testing the model, developed from a consideration of the aforementioned laboratory studies, for its accuracy in describing the decomposition of plant residues in an 8-year field experiment.

Introduction

During the past decade, many simulation models have been developed to describe the cycling of nutrients, especially N, in soils. Frissel and Van Veen[12] have classified soil N models according to their purpose, dynamics and concept. Scientific models which have been developed to better understand the mechanisms of coupling of N transformation processes may be divided into three groups, *viz.* (i) those in which

physico-chemical processes dominate (erosion, transport, volatilization), (ii) those in which the uptake of N by plants and the fate of plant N are emphasized, and (iii) those in which biological, especially microbiological processes, dominate (nitrification, denitrification, mineralization, immobilization). Models of the latter group have ranged in their complexity and level of resolution. For example detailed models have been developed to describe nitrification[27], whereas modelling of N fixation has not been attempted at a detailed level. Simulation models of the growth and activity of the soil biomass in relation to its role in mineralization-immobilization processes are complex[20, 26, 29, 36, 40]. Such models often form the core of models of the total terrestrial N cycle.

Several identical concepts are embodied in the models of McGill *et al.*[26] and Van Veen and Frissel[40], *viz.* (i) the availability of organic C mainly controls the growth of soil (microbial) biomass, which in turn controls N uptake and release, (ii) organic fractions are included to deal with differences in quality and availability of substrates, and (iii) biomass turnover or death is treated explicitly. This concept acknowledges microbial productivity as essential to the dynamics of the terrestrial N cycle. Such models require data on the rates of uptake of organic compounds by soil organisms, on the efficiency of substrate use for biosynthesis and energy supply, and on the nature and rates of release of organic products from the soil biomass.

Here we briefly discuss pertinent aspects of the kinetics of turnover of soil biomass to provide background information on the concepts expressed in the recently modified model of Van Veen and Frissel[40]. This model has been further modified on the basis of laboratory and field data obtained with different Australian soils.

Kinetics of C and N turnover through the biomass

Soil microbial biomass has been called 'the eye of the needle through which all the natural organic material that enter the soil must pass'[17]. Thus the microbial biomass, viewed as an entity, may be regarded as a transformation station, whereby materials are taken up, converted into new products, and subsequently released actively or passively. The processes occur simultaneously and are interdependent, but are described in most models on different levels of sophistication. Uptake and efficiency of use of substrates from solutions have been rather well described, although Bazin *et al.*[3] correctly pointed out the limits of the application of models developed for steady state laboratory cultures to models of soil ecosystems, which are subject to environmental perturbations and so are unlikely to reach true steady states.

Concepts of microbial processes other than those of growth have also been developed from chemostat systems but these are even less readily applicable in descriptions of microbial turnover processes in soils. In chemostat systems those processes which require substrate metabolism but which do not contribute to growth are usually accounted for by a maintenance coefficient (*e.g.* Pirt[30]). Thus maintenance energy is required for the preservation of the correct ionic composition and intracellular pH of cells, for the maintenance of a pool of intracellular metabolites against concentration gradients and for the turnover of cellular constituents, *etc.*[38]. The concept of maintenance energy serves to explain differences between theoretical and experimental growth yields, *i.e.* it serves to balance the substrate budget.

The concept of cell maintenance has been defined for pure cell cultures, and cannot be readily applied to mixed cultures[43]. Further, death and (auto)lysis of cells are not implied, *i.e.* the concept does not express the dynamics of microbial death or of turnover of nutrients through the biomass. It seems therefore more appropriate to model biomass turnover of C and N in soils by using either first order rate kinetics[26, 40] or exponential functions[36] to describe explicitly the formation and release of microbial metabolic products and their availability as substrates for successive populations.

Nevertheless the concept of maintenance has been applied to describe microbial turnover processes in soil[1, 2, 5]. Verstraete and Voets[44] have stated that the turnover rate, r, *i.e.* the amount of biomass C that is replaced per unit of biomass per hour is defined by

$$r = m_e \cdot Y_M$$

where m_e = maintenance coefficient, *i.e.* the amount of substrate C needed per unit of biomass C per hour to maintain the biomass in an active physiological condition, and Y_M = maximum growth yield, *i.e.* the amount of biomass C produced per unit of substrate C. Newman and Watson[28] have applied similar kinetics to describe C turnover through the microbial biomass in the rhizosphere.

Detailed descriptions of biomass turnover in soils are seriously hampered by a lack of knowledge of the mechanisms of microbial death and of other processes by which organic substrates become available for decomposition. Recent studies have emphasized the potentially important role of predation in nutrient cycling. Chemostat studies of predator-prey relationships have been extensively modelled (*e.g.* Curds[7] and review by Fredrickson[10]). and have included those by Hunt *et al.*[15] who developed a model to describe the effect of predation on the amounts of bacterial C, N and P. However, neither hyperbolic kinetics nor the

classic Lotka–Volterra models accurately describe processes even under chemostat conditions[45]. The effects of environmental conditions (*e.g.* thawing of frozen soils, rewetting of dry soils, temperature and moisture stresses) on the death of organisms and on nutrient release, have been included in simulation models[15, 26, 36].

Model development and experimental verification

The iterative process of model development and experimental testing has led to a deeper understanding of complex systems. Models by Van Veen and Frissel have developed through several stages[11]. The first version was based on processes throught to be controlled by the C:N ratios of different organic materials in soils. Both the second and third versions assumed that N transfers were controlled by growth and decay of the microbial biomass; they differed mainly in the treatment of the biomass turnover process. The fourth and latest version differs from earlier models in the following aspects (1) the fractionation of plant and soil organic matter fractions, (2) variations of the C:N ratio of microbial biomass, (3) the effects of drying and rewetting of soils, and (4) the description of biomass turnover.

Fractionation of organic matter

An early model by Van Veen[39] considered that C compounds of crop residues added to soils were the only available substrates for the soil biomass. The exclusion of microbial exocellular products and cellular debris as potential growth substrates resulted in serious discrepancies between model output and experimental results. A later model with improved predictive value included both crop residue components and soil organic matter fractions as microbial substrates[40, 42].

Large differences in the respective turnover rates of specific compounds in soil and in microbial suspensions have been interpreted as being due, at least in part, to a protection in soil of substrates against microbial decomposition. Thus in the model describing long-term effects of cultivation on the organic matter content of virgin grasslands of North America, Van Veen and Paul[41] included the concept of physical protection of organic matter. In order to embody the concept of physical protection into a short-term N model a revised fractionation scheme was proposed (Fig. 1) which was similar to that devised by Juma and Paul[20]. Fig. 1 shows that plant material is considered as three fractions, the most recalcitrant of which is treated as part of a larger fraction containing also unprotected but recalcitrant microbial products. Other unprotected microbial products are relatively decomposable;

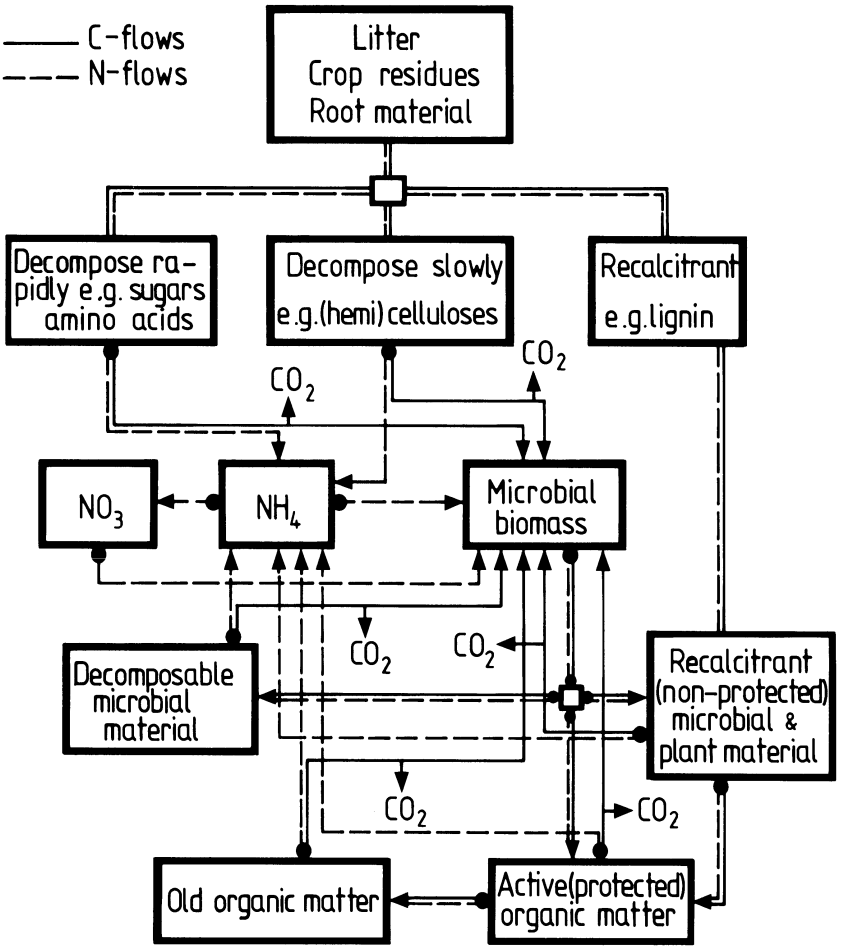

Fig. 1. Scheme (version 4) of the N-mineralization–immobilization model.

some microbial products are protected. In addition to these 'active' fractions, soil organic matter is considered to contain an old inactive fraction, stabilized by unspecified mechanisms or properties.

In proposing a scheme such as in Fig. 1, there is clearly a compromise between the necessity to recognize the heterogeneity of soil organic matter as substrates for microbial metabolism and the impossibility of modelling explicitly the utilization of individual substrates by specific microbial populations. Plant residue components can be adequately determined by existing methods[39]. Hydrolysis of soil organic matter yields old and young fractions, as revealed by radiocarbon dating measurements[24, 25], but it is doubtful whether chemical fractionation techniques alone can satisfactorily be used to estimate the size of biologically important fractions. Recently Richter *et al.*[33] proposed a separation of the labile (young) organic N fraction of soil into three

components, including microbial biomass, based on mineralization patterns under different incubation conditions.

Variable C:N ratio and intracellular composition of the microbial biomass

Several models have used a fixed C:N ratio (usually between 20 and 30:1) of substrate as an index for net mineralization or net immobilization of N[4, 8, 31]. Yet the point at which net mineralization or immobilization of N occurs is not primarily a function of the substrate but rather of the C:N ratio of the newly-formed biomass and of the relative efficiency of C and N utilization[39].

A model accommodating a variable C:N ratio of biomass therefore allows for optimal flexibility[26]. The fourth version of the model assumes that biomass C:N ratios will vary between 5 and 15:1, depending on soil mineral N concentrations. N is considered to limit microbial growth according to hyperbolic kinetics with the saturation constant, $\mathbf{K_s}$ being 1 mg N kg^{-1} soil.

Calculations of the amounts of biomass C and N have been based usually on the assumptions that the C content of cells is approx. 50%, that the C:N ratio is 5–15:1 and that the dry weight of a cell is approx. 20% of the fresh weight. Such data have been used for example in models relating increasing cell numbers to uptake of C or N, or when model outputs are fitted to experimental data on microbial counts.

Table 1. Dry weights of bacteria, fungi and yeasts grown under different temperatures and osmotic stress conditions

Osmotic stress (MPa)	Dry weights of organisms (% of fresh weights) grown at 12°C			21°C			30°C		
	*B	F	Y	B	F	Y	B	F	Y
0.5	15	9	21	16	19	27	15	28	20
2.3	33	29	40	37	35	42	41	41	42
5.2	46	44	45	45	40	47	50	50	47

*B bacteria. F fungi; Y yeasts: Data adapted from van Veen and Den Hartog, in preparation.

However, data shown in Table 1 and obtained in experiments with organisms grown on the surfaces of semi-permeable membranes at different osmotic tensions, demonstrate that such an assumption of the percentage dry weight content of microorganisms is incorrect (Van Veen and Den Hartog, in prep.). Since the percentage of C and N contents of the dry cellular material did not change significantly with the growth conditions, the results indicate that perhaps in soil during conditions of drying, a similar accumulation of C and N per cell may occur

with increasing osmotic tension. Most models correct for the effects of moisture on microbial activity, but none has allowed for the accumulation of cellular organic materials during drying, an effect which could markedly influence the turnover of C, N and other nutrients.

Effects of drying and rewetting of soils

The enhancement of CO_2 evolution and of N mineralization following rewetting of dry soils has long been recognized as an important phenomenon in the processes of C and N turnover (*e.g.* Birch[6]). Underlying mechanisms are not well understood. The enhanced activities are thought to result from the increased availability of organic substrates due either to chemical reactions or to the death of cells during drying[16, 37]. The relative importance of these two possible mechanisms is unknown, and the uncertainty regarding the effect of soil drying on the microbial biomass is reflected in two models which explicitly describe microbial death. In the model of McGill *et al.*[26] death rate is inversely related to moisture stress; death at extreme moisture stress is not considered. In the model of Smith[36] an exponential relationship between moisture stress and death rate is used.

In our model we have included both a moisture effect on microbial death rate, and a stochastic event of moistening a dry soil. Death rates of microbial populations are described by first order rate kinetics; the effect of moisture stress is included by using a reduction factor[40], which may range from 1.0 to 0.0 at moisture stresses from 30 to 8000 kPa. At moisture stresses $>$ 3000 kPa it is assumed that all biological activities cease and microbial death is halted. When soil is remoistened after a period of drought, the moisture content during the first day or less is considered to be optimal for biological activity irrespective of the actual moisture contents. After this period the effect of the actual moisture content on biological activity operates again.

Even when daily data on moisture stress or soil temperatures are known, they are often too detailed to incorporate into a model, which simulates processes over a period of a year or longer. Usually, data averaged over a week or a month are put into the model and intermittent data are calculated by interpolation. The occurrence of wet periods during a dry Austrialian summer was simulated by the use of a random-number generator. Frequency of wetting could be set by a switching function, which had a value of 1 for numbers greater than a set value and of 0 for numbers lower than the set value.

The potentially large effects of drying/rewetting events on the decomposition of organic material is shown in Fig. 2. It should be pointed out that we did not change the values of the rate constants of

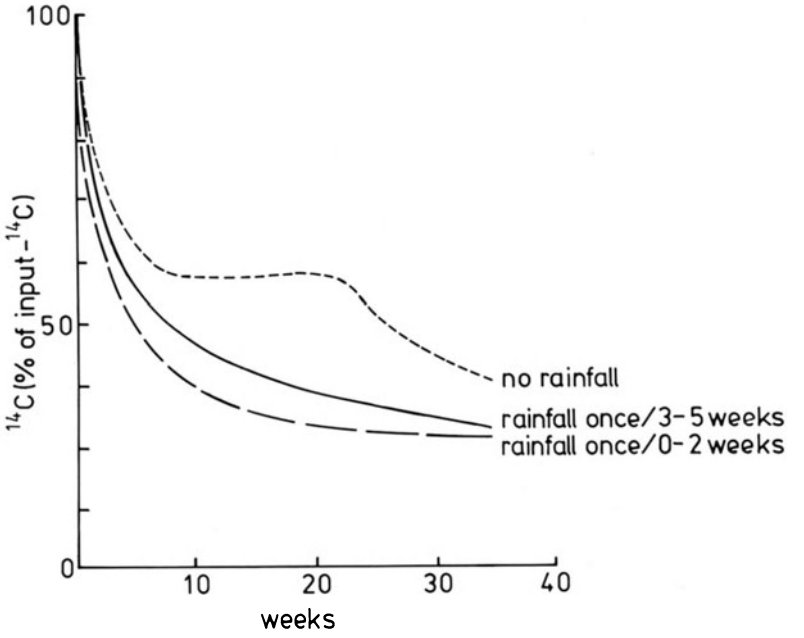

Fig. 2. Calculated effect of frequency of rainfall events (at random) during the dry summer in South Australia on the decomposition of ^{14}C labelled Medick residues.

microbial death or decomposition of organic matter at the occurrence of drying/rewetting events. Field experiments with ^{14}C-labelled crop residues confirm that decomposition continues during the dry summer period in South Australia[22]. The calculated decomposition rate, assuming a rainfall frequency of once every 0–2 weeks, fits with the experimental observations (see section on model verification).

Biomass turnover

Chemostat studies with pure cultures have shown that slow-growing organisms have lower maintenance energy coefficients than fast-growing organisms. Thus Hunt[15] proposed that in soil the microbial population could be considered to consist of an active population with high maintenance energy requirements and an inactive population with relatively low requirements. Also, in the third version of the N model of Van Veen and Frissel[40], the soil microbial populations were grouped according to their function, which determined not only the growth rates of the respective groups, but also the death rates (which were directly related to the growth rates). However, the model was unable to describe sudden changes in populations *e.g.* following a high substrate input, or the effects of soil properties in influencing the proportions of organic residues accounted for in biomass.

Ladd *et al.*[21,22] observed that following the metabolism in calcareous soils of glucose plus $^{15}NO_3^-$ or of ^{14}C, ^{15}N-labelled legume material, those soils of higher clay content generally retained higher proportions of residual organic ^{14}C and ^{15}N as labelled biomass. This difference between soils remained apparent even after eight years decomposition of labelled plant material in the field. Therefore the turnover of nutrients through the soil biomass is influenced not only by the physiological states of soil organisms but also by soil properties, including perhaps soil texture. The effects of soil on biomass may be on biosynthesis efficiency and/or on death rates. There appears to be no direct evidence to support the notion that clay content specifically leads to greater efficiency of substrate use by growing microbial populations. Soil properties other than clay content could have been responsible for a greater efficiency of growth in those soils were higher proportions of organic residues were accounted for as biomass. It is known for example that the efficiency of C utilization by microorganisms is dependent on the nature of the N source[38] and it decreases when other nutrients are lacking[32]. However, in the cases of the field soils concerned[22] the evidence suggested that N at least was in adequate concentrations, since net mineralization of added plant N occurred throughout the experimental period.

The alternative explanation that soil properties, specifically soil texture, affect the overall death rate of the soil biomass, is supported by some independent evidence. Roper and Marshall[34,35] showed that colloidal particles *e.g.* montmorillonitic clay, protected coliform bacteria in saline suspensions from predation by bacteriophages and amoebae. Also, transmission electron micrographs of soil have revealed clusters of microorganisms surrounded by clay particles[9], providing illustrations of relatively closed microbial communities. Such communities may be protected from predators or from the rigors of harsh and changeable environments. Organic substances released from cells by autolysis, are for the greater part likely to be retained within the micro-environment of the survivors of the community until utilized for new cell development.

A four-year field study of the decomposition of ^{14}C and ^{15}N-labelled plant residues in soil has shown that isotope-labelled biomass accounted for larger proportions of labelled residues, the smaller the amount of plant material added initially[23]. These observations are consistent with those obtained in a laboratory experiment in which ^{14}C-labelled bacteria were added in different amounts to soil, and incubated for 115 days. At that time, and for the lower input treatment, labelled biomass ^{14}C accounted for 19% of input ^{14}C and 39% of residual organic ^{14}C.

For the higher input treatment where three times as much ^{14}C was added initially, the respective values for labelled biomass were 16% and 32% (Van Veen *et al.*, in preparation).

These results have led to a changed description of microbial death from that presented in the third version of the model. We now consider that just as organic matter may be considered as protected and unprotected residues, so too is the soil biomass. In the new model we propose that each soil has a specific capacity to preserve or protect microorganisms. This approach differs from that describing the protection of non-biomass organic residues in which the size of the protected portion is considered to be a fraction of the total amount and not an absolute value. In the model, if the total soil biomass $\leqq$ to the preservation capacity of the soil, the entire microbial population is considered to be protected. The death rate of protected organisms is set rather low, 0.5% per day. Biomass formed in excess of a soil's preservation capacity is assumed to decay at a much higher rate, about 70% per day. The 'closed' system formed by protected microorganisms and organic substrates released from autolysing cells is effected in the model by assuming that most of the C and N flow from the biomass enters the decomposable and recalcitrant microbial residue fractions (Fig. 1) and is retained within the community, and that only a minor portion flows to other compartments. The magnitude of these fractions depends on soil type.

The capacity of soil to preserve biomass is seen to be a key parameter for the description of C and N turnover in soil. Fig. 3 illustrates the dependence of the size of the biomass pool on the preservation capacity of a soil. The results presented are of a case study to determine the effect of the magnitude of the preservation capacity (set at 150, 250 and 350 μg biomass C g^{-1} soil) on the total biomass C of a sandy soil

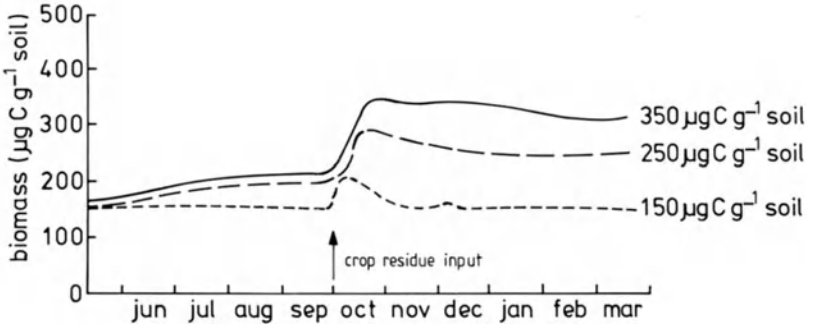

Fig. 3. Calculated effect of the protection capacity of a sandy soil (Roseworthy, South Australia) on the total biomass C content after addition of Medick crop residues.

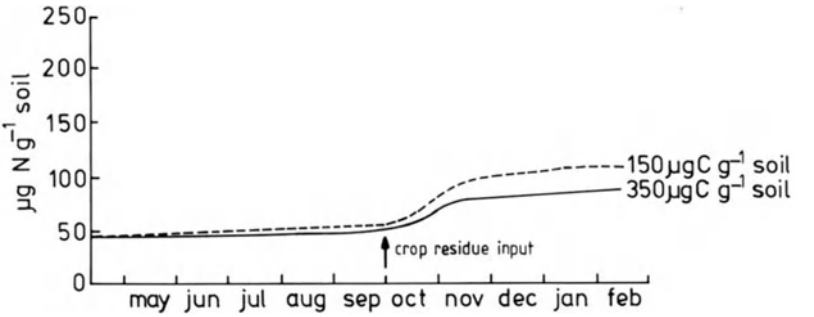

Fig. 4. Calculated effect of the protection capacity of a sandy soil (Roseworthy, South Australia) on the N mineralization after addition of Medick crop residues.

amended with legume residues. Fig. 4 illustrates the effect on the mineralization of N from such an amended soil. To data we have assumed that the capacity of a soil to preserve biomass is roughly equal to the size of the biomass of the soil, given no recent soil disturbance or accession of organic substrates.

Model performance and experimental testing

The above concepts of biomass turnover were tested and developed further in laboratory experiments in which isotope-labelled plant material, bacteria and defined substrates (glucose, NH_4^+, PO_4^{3-}) were separately incubated in a clay and a sandy soil. Both soils were from South Australia, the clay from Northfield and the sandy soil from Roseworthy. The soils were incubated mostly at 40% of their respective water-holding capacities but treatments involving intermittent drying and rewetting of soils were introduced during the later stages of the incubation. Details of $^{14}CO_2$ production and of changes in the amounts of inorganic ^{15}N and ^{14}N, of labelled residual organic C and N, and of labelled and unlabelled biomass C and N will be presented elsewhere (Van Veen *et al.*, in preparation). Here we discuss some of the data from the glucose-amended soils only since these data have formed the initial basis for model development and testing.

Rates of $^{14}CO_2$ production accompanying ^{14}C-glucose decomposition were considerably higher in the sandy soil compared to the clay soil; for each soil exponential rate constants declined with incubation time but those for the Roseworthy sandy soil remained consistently twice as high as those calculated for the Northfield clay soil (Table 2).

Biomass ^{14}C was determined by the chloroform fumigation technique using a **k** factor of 0.45[19]. It was assumed that the factor remained

Table 2. $^{14}CO_2$ evolution from a sandy and clay soil incubated with ^{14}C-glucose

	Soil	Decomposition time (days)				
		3	10	24	38	59
CO_2–^{14}C production (% of input ^{14}C)	Northfield clay	29.7	35.3	39.7	42.3	45.0
	Roseworthy sand	37.6	46.3	53.1	56.8	60.3
*k X 10^{-3} (day^{-1})	Northfield clay	30	8	5	3	2
	Roseworthy sand	40	15	10	6	4

* k, exponential rate constant $= -\ln (N/N_0)/t$ where N = ^{14}C remaining at the end of a decomposition period t (days), N_0 = ^{14}C at the beginning of a period.
Data adapted from van Veen *et al.*, in preparation.

Table 3. Biomass ^{14}C in a sandy and a clay soil incubated with ^{14}C-glucose

Biomass ^{14}C	Soil	Decomposition time (days)				
		3	10	24	38	59
% of input ^{14}C	Northfield clay	44	39	37	34	32
	Roseworthy sand	27	22	20	18	16
% of residue ^{14}C	Northfield clay	62	60	61	59	58
	Roseworthy sand	43	41	42	42	39

Data adapted from van Veen *et al.*, in preparation.

constant for both soils and for different incubation times, although it is appreciated that the factor is likely to differ for protected and unprotected biomass. Biomass ^{14}C as a percentage of input ^{14}C decreased with time in each soil, and at a relatively faster rate in the sandy soil (Table 3). However, as a percentage of residual ^{14}C, biomass ^{14}C in each soil remained remarkably constant over the entire incubation period. Labelled biomass of the sandy soil was about one half that of the clay soil, and unlabelled biomass about one third that of the clay soil. Neither biomass measurement reflected rates of mineralization of glucose C in the two soils.

Initially the model was tuned to two sets of data obtained with the Northfield clay soil, *viz.* $^{14}CO_2$ evolution and biomass-^{14}C as a percentage of input ^{14}C. The data were put into the model as a table from which a curve was computed by linear interpolation to facilitate the comparison of experimental results and model output (Figs. 5a, b, c and 6b, c). Tuning concerned (i) the establishment of values for the preservation capacity of the soil and for the fraction of microbial products which flowed from and hence became unavailable as substrates for the cells, and (ii) the establishment of the most appropriate kinetics for cell growth.

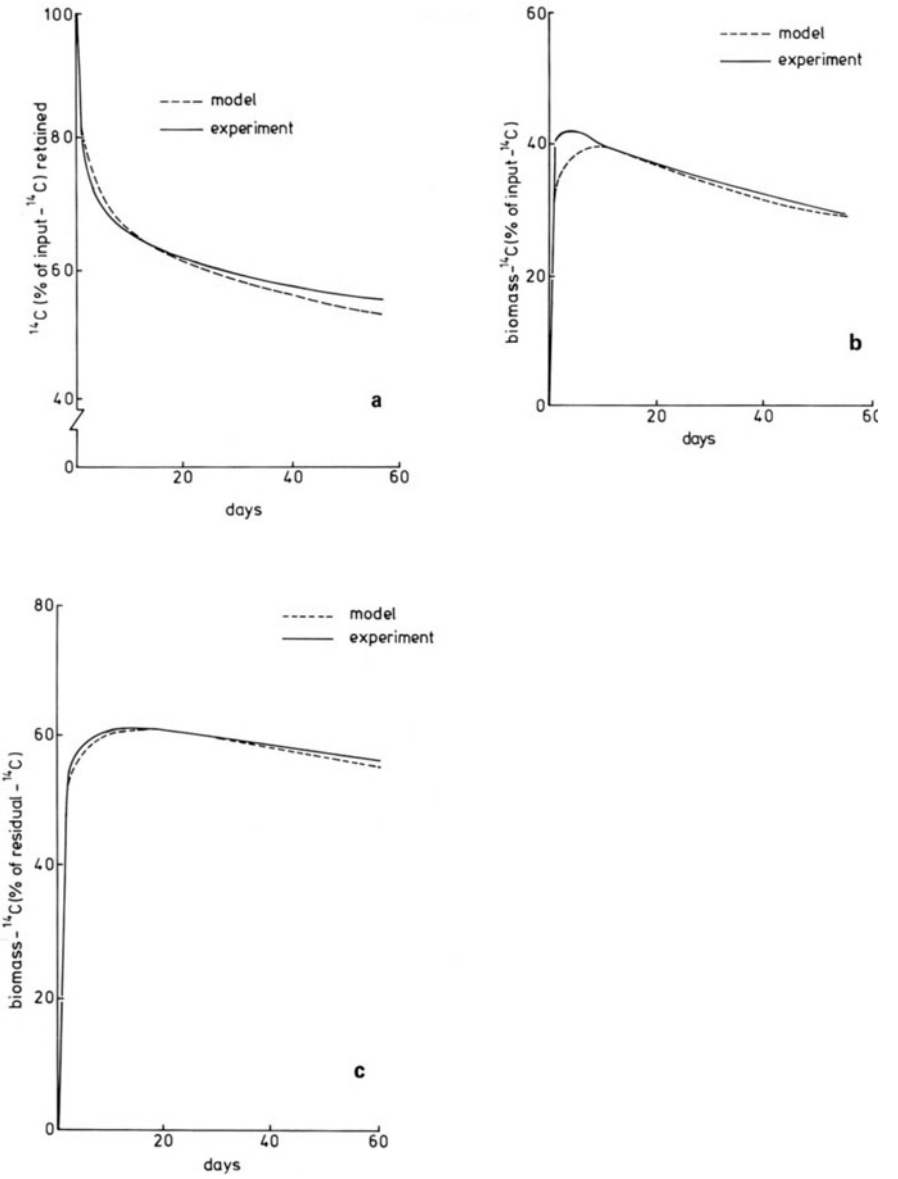

Fig. 5. Calculated and experimentally observed data for incubation of ^{14}C-glucose with a clay soil (Northfield, South Australia) at 25°C and 40% WHC. a Loss of $^{14}C-CO_2$; b Formation of ^{14}C biomass as % of input; c Formation of ^{14}C biomass as % of residual ^{14}C.

The value of the preservation capacity of the Northfield clay soil was set at 700 μg biomass C g^{-1} soil. Model performances with hyperbolic kinetics were slightly better than with first order rate kinetics and were, therefore, applied. The value of the parameter controlling the

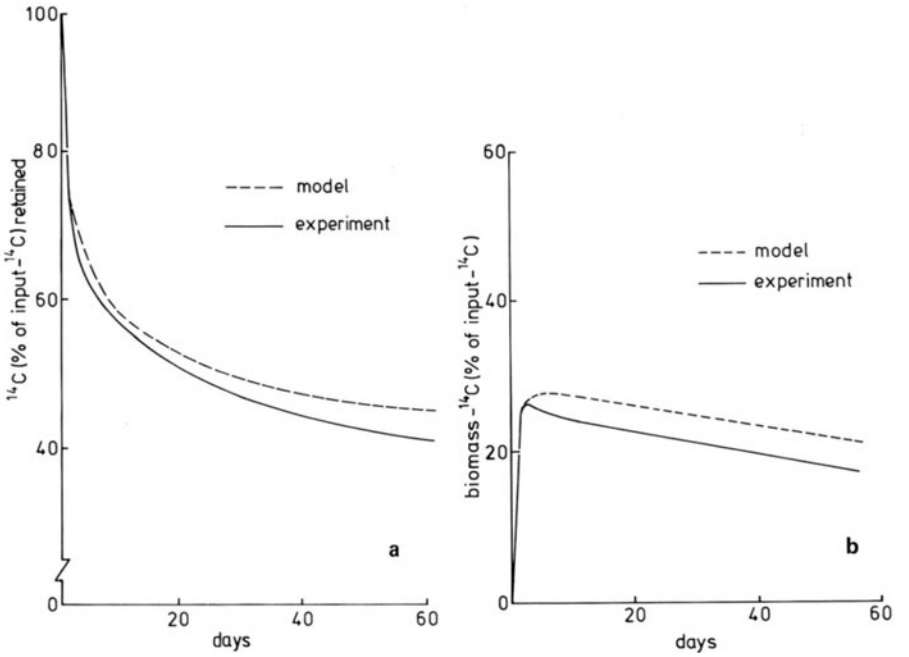

Fig. 6. Calculated and experimentally observed data for incubation of ^{14}C-glucose with a sandy soil (Roseworthy, South Australia) at 25°C and 40% WHC. **a** Loss of $^{14}C-CO_2$; **b** Formation of ^{14}C-labelled biomass.

fractionation of microbial products into the protected pool and the unprotected, decomposable and resistant pools appears to have a substantial influence on model performance. Thus the extent to which available carbonaceous microbial products are retained within the vicinity of surviving organisms may be an important issue in the dynamics of C in soils. For the Northfield soil, the proportion of microbial products which 'leak' from the microbial communities and which flow to protected organic fractions was set at 10%.

A good fit was obtained between experimental results and model output (Figs. 5a, b, c). The manner in which the model was tested, by using several sets of experimental data, tended to avoid the achievement of a good fit due to one error offsetting another. Further, small discrepancies in observed and calculated values for $^{14}CO_2$ production, and biomass-^{14}C as a percentage of ^{14}C input, could have resulted in large discrepancies in the observed and model output values for biomass ^{14}C as a percentage of residual ^{14}C.

The model developed for the clay soil was further tested as a description of ^{14}C-glucose decomposition in the Roseworthy sandy soil. Figs. 6a, b compare model outputs and experimentally observed values for respectively, $^{14}CO_2$ production and biomass ^{14}C as a percentage of ^{14}C

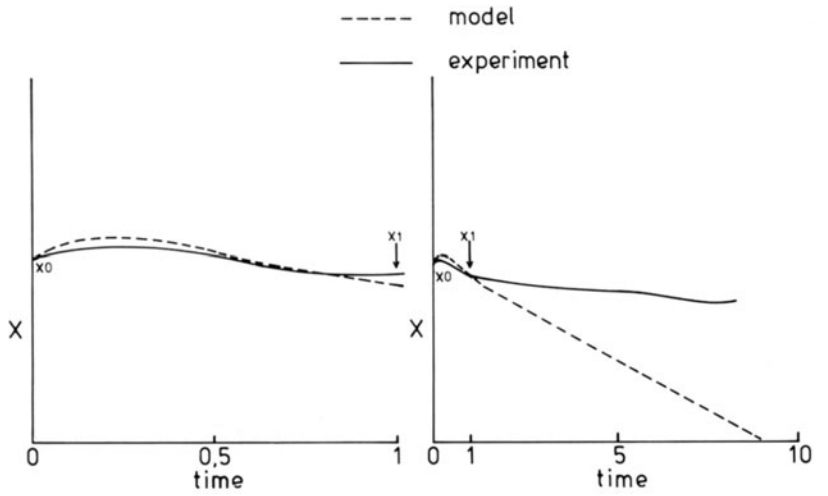

Fig. 7. Illustration of a clearly wrong prediction of the fate of a fictive variable X over a long period (0–10) with a model that satisfactorily predicts the fate of the same fictive variable X over a short period (0–1).

input. The results are preliminary. As yet, only the value for preservation capacity of the soil has been altered in the model (from 700 to 300 μg biomass C g^{-1} soil). Nevertheless the trends from the model output clearly approximated those of the experimental data and suggest that the measured differences in the fate of C metabolized in the clay and sandy soil may be largely explained by the differences in the extent to which the two soils were able to protect or preserve biomass.

The best ways to test models have long been a subject for discussion amongst modellers (see for example Frissel and Van Veen[11]). Surprisingly little attention has been given to assessing the performances with time of those models primarily developed to describe processes over relatively short terms. Models which acceptably predict changes in a time variable over say a growing season, may prove to be clearly inaccurate over a decade as illustrated in Fig. 7 for a fictive variable X; those models which appear to be accurate in describing events over a decade may be unsuccessful in predicting changes over a century (see for example Jenkinson[18]) on the limited value of a simple exponential model to describe long term turnover of soil organic matter.

The model used to describe glucose decomposition in the laboratory-incubated soils has been tested with data from an eight-year field experiment in which ^{14}C, ^{15}N-labelled legume material has been decomposed in Roseworthy soil (Ladd *et al.*, in preparation). Data for residual organic ^{14}C and ^{15}N and for biomass ^{14}C and ^{15}N for the first four years have been published by Ladd *et al.*[22].

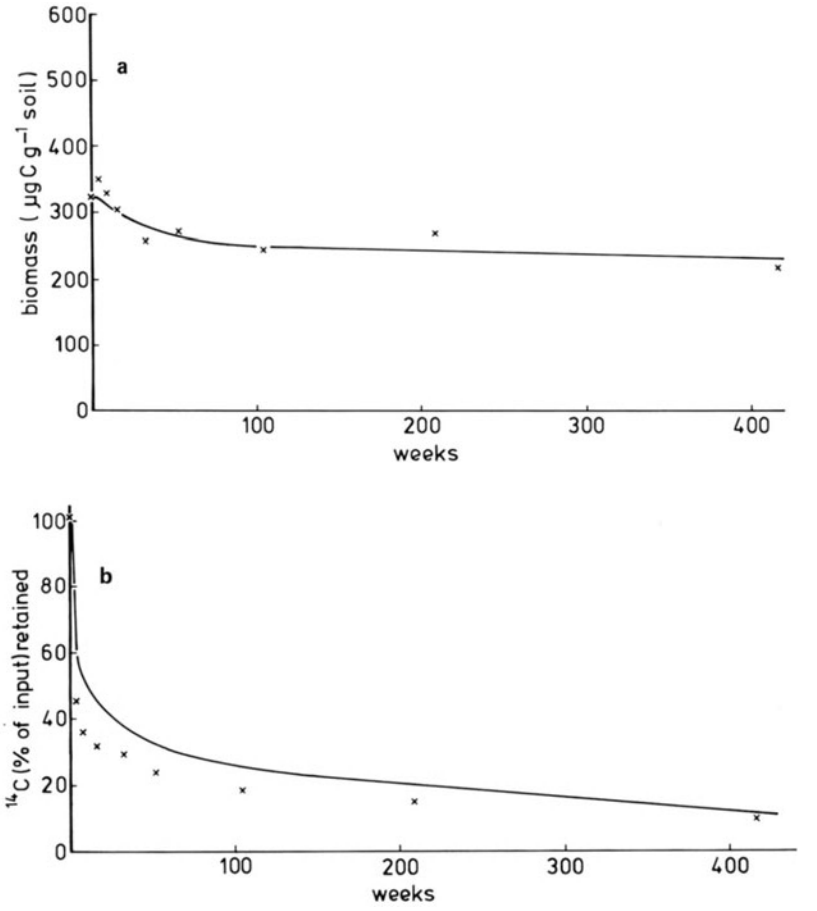

Fig. 8. Calculated (——) and experimentally observed (X) data for a single addition of ^{14}C labelled Medick crop residues to a sandy soil (Roseworthy, South Australia) under field conditions. a Biomass-C; b ^{14}C–CO_2 loss.

In earlier versions of the model, biomass often showed an anomalous pattern comparable with the example shown in Fig. 7. However, the present model accurately predicts total biomass-C changes over the period of eight years (Fig. 8a). Fig. 8b shows the corresponding model and experimental plots for the loss of ^{14}C–CO_2 as the legume material decomposed. The pattern is similar to that shown in Fig. 6a for glucose decomposition over a shorter period. In both cases the CO_2 loss is greater than the calculated one. Better fits may be obtained if some tuning of other parameters than the preservation capacity had been introduced and if we have a better recognition of differences between substrates, *i.e.* glucose *vs* legume material.

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Dynamics of organic matter in soils

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Key words Biomass dynamics Carbon Nitrogen Nutrient management Soil organic matter

Summary Dynamics of C, N, S, and to some extent P are expressed by a knowledge of the size and turnover rates of plant constituents such as soluble C and N components, cellulose and hemicellulose, and lignin. Soil organic matter constituents include: the microbial biomass as determined chemically or microscopically, non-biomass active components determined by isotopic dilution, stabilized N constituents for which good techniques are not yet available, and resistant or old C and associated N determined by carbon dating. The processes involved in the nutrient transformations and transfers are reasonably well understood. The control mechanisms require further elucidation to be able to extrapolate from the laboratory to the field, and between field sites. Major control mechanisms requiring further insight include the effects of C availability on transformations of C and N. The other control for which every little is known is that of spatial compartmentalization. Compartmentalization ranges from landscape or management sequences to pedogenic layers, rhizosphere-mycorrhizal effects, clay-sesquioxide surfaces, aggregation, localized enzymes, and microbial effects such as membrane boundaries. Control mechanisms for concurrent mineralization-immobilization, the stabilization of microbial products, and the relative role of the biomass as a catalyst rather than as a source-sink for nutrients, must be understood. There is potential for combining a knowledge of microbial production and turnover with that of the roles of the soil organic active fraction as a temporary storehouse for nutrients. This, in conjunction with management techniques such as zero tillage and crop rotation, should make it possible to better utilize soil and fertilizer N, especially in areas of the world where the cost of nutrients is high relative to the value of the crop grown.

Introduction

Soil organic matter (SOM) dynamics play a major role in natural ecosystems and extensive agriculture. In intensive agricultural systems with high fertilization rates, the various organic components have the potential for acting as a temporary nutrient reservoir. The proper management of this reservoir should make it possible to increase the efficiency of use of both soil and fertilizer nutrients. The net magnitude and general microbiology of processes such as N, P, and S mineralization-immobilization are reasonably well understood. However, information is lacking concerning the actual or gross flows that occur in the concurrent processes of mineralization-immobilization. The *in situ* controls also require further investigation if they are to be utilized in a nutrient management program.

Empirical measurements of the transformations of C, N, S, and P cannot be determined for the many soil–plant type interactions involved in agricultural and non-agricultural systems. Extrapolation of the available information from the laboratory and the few field sites where detailed information is available[16,41,53] requires a better knowledge of the factors controlling the various processes[29]. Although C, N, S, P, and the minor elements are involved in SOM dynamics, because of space restrictions, the rest of this paper will stress C and N interactions.

A reasonable amount of information is now available on abiotic controls such as moisture and temperature, aeration, and composition of the plant residues[31,32,34]. Not enough information is presently available to relate the effects of soil spatial compartmentalization and C availability to SOM dynamics. Spatial compartmentalization includes effects such as catenary sequences, pedogenic layers, soil structure and aggregates, rhizosphere-mycorrhizal effects, clay or sesquioxide surfaces, localized enzymes, and microbial membrane boundaries.

C available for microbial growth, whether from resistant humic components, plant detritus, or as photosynthate translocated to symbiotic partners, plays a major role[47]. The recent advances in the identification of soil humate constituents[5,50] and advances in NMR spectroscopy[12,42] should be related to kinetic studies of SOM. The increased understanding concerning the flow of plant C to underground structures such as mycorrhizal fungi and N-fixing organisms[19,54] also is aiding in the interpretation of underground dynamics.

Organic matter dynamics relative to soil type

The type, content, and extent of aggregation of clay continues to be recognized as a major controlling factor affecting SOM dynamics[52]. Bacterial utilization of proteins in the presence of clay minerals[25] could be divided into three types. At high protein-to-clay ratios, growth was not affected by the clay. At intermediate ratios, growth rate but not the final yield was reduced, and at low protein-to-clay ratios of montmorillonite and kaolinite, the protein was unavailable for hydrolysis. Clay minerals have been shown to protect microbial metabolites produced during degradation of labelled substrates[20,29].

The strong association of minerals such as allophane with SOM components makes this a useful tool for measurement of clay–SOM interactions. Studies on the addition of ^{14}C-labelled glucose, cellulose, and proteins from algae and of labelled polysaccharides from wheat straw[26,58] showed that the allophane caused a major reduction in the

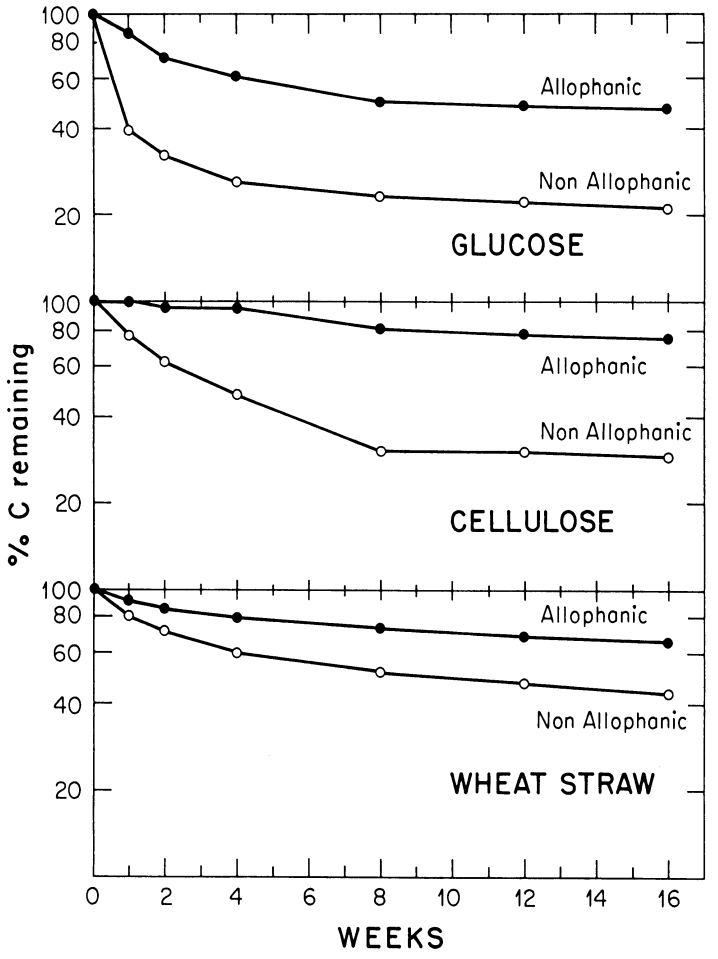

Fig. 1. Comparison of degradation of wheat straw, cellulose, and glucose in allophanic and non-allophanic soil (Zunino *et al.*[58]).

degradation rate of added carbonaceous materials (Fig. 1). The strong initial protective effects on compounds that include glucose and cellulose must lead to further investigations on the effects of possible inhibition of microbial activity during the initial degradation and in the sequential growth phases, in addition to the effects involving microbial metabolite protection. Increased efficiency of microbial growth[46] in the presence of allophanic clays have been cited. Microbial growth on simple compounds such as glucose and proteins usually shows high efficiency values in soils; these are very similar to those obtained in pure culture[8,36]. The work of Marshman and Marshall[25] also showed no effects of clays on microbial growth efficiency.

Table 1. Radiocarbon age of Melfort soil and fractions collected in 1978

Fraction	Proportion of organic C	Age, % modern	Eq. Age, years
Soils	1.00	90.6	795
Coarse silt	0.25	90.6	800
Fine silt	0.29	88.7	965
Coarse clay	0.31	85.5	1255
Fine clay	0.08	97.9	170

Not dated: Organic carbon with sand (0.03).

Work with tracers has verified that large amounts of labelled phenolic compounds are incorporated into stabilized humus, while non-aromatic materials are incorporated into the soil biomass[18]. The addition of ^{14}C labelled melanic fungal residues[24] resulted in a slow degradation rate of the materials and concentration of the cell wall materials in the humin fraction of SOM.

The stabilization by fine clays, of otherwise readily decomposable substrate, is also shown by data from C dating (Table 1). The differences in the C age between the fine clay and the coarse clay of this soil is probably associated with differences in their clay mineralogy. The protection of otherwise readily degradable substrates, including proteinaceous materials and polysaccharides[3], makes clay adsorbed materials an important contributor to the dynamics of materials with an intermediate range of turnover. The association of very old materials with coarse clays and silts must be attributable to a different type of chemical bonding with recalcitrant humates and more resistant microbial cell wall constituents[30].

The use of flotation to separate light and heavy fractions differentiates plant residues and some light aggregates[45], and is proving a useful separation technique for both forest and agricultural conditions[10,48]. Tisdall and Oades[52] have reviewed the literature on mechanisms of aggregate formation. Data on the actual effect of protection on degradation rates are, however, still hard to obtain.

The molecular weight distribution of SOM is altered during microbial attack and humification. Incubation of plant litter is said to result in a decrease in the amount of SOM in the greater than 100,000 molecular weight range; incubation of the soil alone showed the opposite effect[57]. These observations fit in with other suggestions[50] that the initial microbial breakdown of plant residues is followed by the synthesis of humic substances of high molecular weight. Ruggiero *et al.*[42] have shown with NMR spectroscopy that incorporation of non-aromatic molecules such as polysaccharides into higher molecular weight fractions is attributable to the trapping of non-humic substances

in the voids of the high molecular weight polymer. Anderson[2] had postulated that humification leads to a decrease in molecular size, *i.e.* the production of fulvic from humic acids. However, fulvic acids usually show high tracer activity and turnover rates. This anomaly was explained when Anderson and Paul[4] showed that elimination of the highly-labelled, low molecular weight extraneous materials present in what is normally known as fulvic acids, by dialysis through a 2,000 molecular weight size membrane, results in fulvic acids that are as old as humic acids in this soil.

The role of the active fraction in SOM dynamics

The suggested occurrence of an active SOM fraction[9] was quantitatively developed[14] with ^{15}N and kinetic analysis. This fraction has more recently been divided into a biomass and non-biomass active component[35]. The measurement of NO_3–N accumulated over long-term aerobic incubation of soils[49] has led to the calculation of a potentially mineralizable N pool (N_0). This has been a very useful concept for determining the effect of controlling factors such as temperature[33], moisture, and organic matter[40] on mineralization rates.

Tracer studies have indicated that recently immobilized N is incorporated into a series of soil fractions such as humic and fulvic acids and ^{14}C and ^{15}N components that can be separated by acid hydrolysis or particle size analysis. No one fractionation technique has been found to adequately separate SOM into biologically meaningful components. Paul and Juma[35], in their simulation model, found that a realistic description of N cycling in soil should consider at least four separate pools: biomass N, non-biomass active N, stabilized N, and old N (Table 2).

The chloroform-incubation technique is useful for following tracers through the biomass as well as determining its relative size and nutrient content. The determination of a K_N value representative of the proportion of biomass N mineralized after fumigation has been based on ^{15}N

Table 2. N pools and techniques used for pool size determination in a Chernozemic soil*

	% of soil N	$T_{1/2}$ yrs	Method of determination	Relative contribution of mineralization %
Biomass	4–6	0.5	Fumigation incubation	30
Active non-biomass	6–10	1.5	Isotope dilution	34
Stabilized	36	22 yrs	By difference	35
Old	50	600 yrs	Associated with old C (carbon dating)	1

* During 12 week incubation.

Table 3. Parameters for the net mineralization of glucose and straw-^{14}C during 7 years incubation of Sceptre soil in the field (Voroney[55])

Glucose-^{14}C remaining (%)	=	$29e^{-.36t}$ +	$40e^{-.023t}$ +	$31e^{-.0002t}$
$T_{1/2}$ (days)		2	30	3450
Straw-^{14}C remaining (%)	=	$52e^{-.036t}$ +	$19e^{-.003t}$ +	$29e^{-.00026t}$
$T_{1/2}$ (days)		19	230	2653

e = base of natural logarithm; t = time in days.

labelled organisms added to soils[27,28] and the chloroform treatment of an *in situ* soil population of labelled organisms. The effect of varying rates of N immobilization after fumigation of biomass with different C:N ratios was overcome by using a sliding scale of CO_2–C:NH_4^+–N produced during incubation of different soils to correct the K_N value for immobilization[56].

Table 3 shows the first-order equations for net mineralization (no correction for microbial growth) of glucose-^{14}C and straw-^{14}C remaining in soil after 7 years' field incubation in a semi-arid climate[55]. Glucose-C was utilized with a high efficiency as the first component degraded represents 29% of the added glucose-C, with a half-life of 2 days. The intermediate fraction with a half-life of 30 days accounted for 41% and represents degradation of microbial biomass and metabolites. The remaining 31% of the ^{14}-C was present in a stabilized fraction that had a half-life of 3450 days. The equation for decomposition of straw-^{14}C shows that the soluble materials, cellulose and hemicellulose[39], had a net decay rate equivalent to a half-life of 19 days. The amount of straw-^{14}C remaining in the stabilized period is similar to that of glucose-^{14}C, but the glucose-derived C is more resistant than the plant-derived ^{14}C. Some of the straw components such as lignin are probably disappearing faster than the microbial materials derived from the glucose[53].

Tracer data from a number of experiments show an interesting relationship which I shall refer to as the catch-up effect. The long-term degradation rate of materials under one set of environmental conditions appears to be primarily controlled by the particular plant composition and by the stabilization rates within a soil. Factors such as fallow vs. crop, or wet years, often tend to be evened out over extended periods. Although cropping, which drys out the soil, results in lower decomposition rates[44], this is usually counterbalanced by faster rates of decomposition during the fall or following spring under prairie conditions. The 'catch-up' effect involving the interaction of inherent decomposability and stabilization with seasonal effects had not yet been adequately incorporated into the modelling of SOM dynamics.

Interpretation of the role of the biomass and active fraction in nutrient cycling and crop management

Knowledge about the size and turnover of the components constituting the active fraction can be used in the interpretation of nutrient cycling parameters, such as (1) the role of SOM in ecosystem functioning, (2) the interpretation of soil tests for nutrient availability as a prerequisite to fertilization, (3) management to increase the efficiency of fertilizer, symbiotically fixed, and soil N, and (4) the interpretation of information on N and other nutrient dynamics relative to internal cycling and plant uptake (the priming effect).

The SOM active fraction size and turnover rate is related to agricultural practices[21,22,51], and soil vegetation type[3,13]. Adams and Laughlin[1] found biomass C and N of grassland soils to be greater than those of arable soils. Zero tillage, in which plant residues are left on the soil surface, results in an increased concentration of biomass at the surface[6,7], but the overall biomass throughout the rooting zone may not be greatly affected[23,38].

The biomass N, which is a major component of the active fraction, has an ^{15}N content similar to that of the mineral N produced upon incubation, and can play a major role as a source of mineralizable N. Marumoto *et al.*[28] found that oven-drying at 70°C approximated the $CHCl_3$ treatment in its effect in releasing P and N during subsequent incubation. It was concluded that the amount of soil biomass might be used for estimating the mobile plant nutrient pool in soil. Voroney*, in the analysis of N_0 for 100 medium textured soils in Saskatchewan found that the N_0, ranging from 50 to 300 $\mu g\, g^{-1}$ soil, equalled 3.3 times the flush of N produced during incubation after chloroforming. The K_N value for such soils is usually 0.3, indicating that N_0 approximated the size of the biomass.

The internal cycling of N through a large biomass can have major effects on the interpretation of nutrient cycling and fertilizer uptake experiments[15]. An example of the potential for altered ^{15}N effects is shown in Table 4. A nitrification inhibitor added to a soil with added ammonia or urea did not affect plant uptake (data not shown) and had no statistically significant effect on the size of the N biomass. The ^{15}N remaining in the soil after growth of a crop was increased by the presence of the inhibitor from 42% to 57% of that added, and the biomass ^{15}N was doubled. The N mineralized on incubation after crop growth was not affected. The uptake of $^{15}NH_4$ by the

* Personal communication.

Table 4. Effect of nitrification inhibitor on ^{15}N distribution biomass N and net N mineralization after growth of a wheat crop in Chernozemic soil with 0.44% total N content (Juma and Paul[17])

Treatment	^{15}N distribution %			^{14}N $\mu g\ g^{-1}$ soil	
	Plant uptake	Soil	Biomass	Biomass N	Mineralized*
N**	39	42	16	140	96
N + Inhibitor	36	57	26	168	73

* During 12-week incubation. ** N added as ammonia or urea.

microorganisms resulted in a consequent release of ^{14}N. Utilization of this ^{14}N by plants would result in apparent increased utilization of soil N. Acceptance of the significance of internal cycling will result in the recalculation of some tracer data; it should also add to the usefulness of the results in that better estimates of what actually is happening in SOM dynamics can be made.

Anaerobic incubation of soils has been found to be a useful technique for the measurement of N turnover relative to forest productivity[37]. Much of the N released during this period probably can be attributed to biomass N, *e.g.* fungal materials which decay under the anaerobic conditions.

The turnover rates of the biomass are as significant as the sizes of these components. Tropical and temperate soils have similar amounts of SOM, especially when the concentrations in lower portions of the profile are taken into account[43]. However, the former have a higher N supplying power per unit of total N[11]; this must be related to the size and turnover of the biomass and other portions of the active component of SOM.

There is the potential for better nutrient management through techniques such as zero-till intercropping with or without legumes, improved soil and fertilizer efficiency, and better management of harvested areas in forestry[21,43]. Realization of this potential will, however, require a much better application of presently available and of new information on SOM dynamics.

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Concepts of soil organic matter transformations in relation to organo-mineral particle size fractions

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Key words Carbon Concepts Cultivation effects Nitrogen Nitrogen-15 Organic matter models Particle size fractions Phosphorus

Summary Changes in the composition of organo-mineral particle size fractions as a result of cultivation of a grassland soil are discussed with reference to models of soil organic matter formation and turnover. The data presented indicated that physically stabilized organic matter is an important reservoir, with an intermediate turnover time, which is responsible for nutrient supply in agricultural soils. Possible mechanisms of stabilization and mobilization of organic matter are presented in the light of the arrangement of organic and inorganic components of the soil.

Introduction

During the development of soils, organic matter is accumulated through the formation of biomass and organic detritus. In many ecosystems equilibrium levels of soil organic matter are determined by the balance of production of biomass, stabilization of detritus and mineralization (respiration) of organic materials. When this balance is disturbed by the introduction of agriculture or changes in cropping systems which alter the patterns of primary production as well as organic matter stabilization and loss, soil organic matter contents are changed.

Many areas of the Western Great Plains of North America have only come under cultivation within the last 100 years, and during this relatively short time of cultivation, soil organic matter contents have changed substantially from the native prairie levels[13, 39]. These prairie soils therefore afford an opportunity to study the dynamics of soil organic matter in the transition from a native to a cultivated ecosystem, supplementing information on the effects of different cropping systems in Europe[17, 31]. The present review focusses on the dynamics of soil organic matter during the first 60 years of cultivation of Chernozemic soils in Saskatchewan and relates the observed changes in elemental composition, particle size distribution and physical arrangement to conceptual models of soil organic matter turnover.

Evidence for the processes involved in organic matter transformations

Simple models of soil organic matter transformations are usually based on a minimum number of conceptual components such as biomass, labile, moderately labile and stabilized fractions, which turn over at different rates under the control of external factors such as moisture and temperature[32]. Different constituents of biomass such as roots, shoots, fungi and bacteria may be distinguished and some basic chemical properties such as C/N ratios may be incorporated into a model in order to follow of C and N fluxes through the soil system[24]. These models provide mathematical frameworks capable of predicting organic matter levels in different environments and under different management systems[31].

In contrast to such abstract conceptual ecosystem models, other more mechanistic (but frequently non-quantitative) models are based on actual fractions such as humic and fulvic acids and mineral associated humins[1]. This traditional fractionation of soil organic matter, though, has severe limitations as a basis for model construction since the fractions isolated are functionally heterogeneous. Fulvic acids, for instance, may consist of both precursors and degradation products of other humic materials[1]. The similarity in chemical composition of humic acids, humins and the total soil organic matter, as shown by ^{13}C NMR[34] further indicates that these fractions may not be functionally distinct.

This paper attempts to reduce the discrepancies between the requirements of biological or chemical models and the capabilities of organic matter fractionation techniques by considering the possible mechanisms of organic matter input, stabilization and loss in the soil system.

Organic matter inputs

Sources of organic matter inputs are: (1) relatively coarse plant debris that is deposited within or upon the soil, and (2) colloidal and soluble organic compounds from root exudates, microbial products or litter leachates.

Fractionation of soils into particle sizes distinguishes organic fragments $>5\,\mu m$ from colloidal and microbial materials $<0.2\,\mu m$. The fine, colloidal organic matter is characterized by a narrow C:N ratio, high aliphaticity and hydrolyzability indicative of recent microbial products[2]. Organo-mineral size fractions $>5\,\mu m$ contain numerous plant fragments in various stages of decomposition and in association with mineral particles[37].

Particulate organic matter entering the soil from roots or litter is initially colonized by the microbial population and at the same time absorbs mineral particles and metal ions[29]. Roots in particular show extensive mineral associations, and may act as binding agents for large soil aggregates while still functional[41]. Particulate litter accumulates in grassland and pastures but is depleted under cultivation[30]. When grasslands are broken large amounts of organic particles are introduced into the soil, and with continuing cultivation these are distintegrated and their residues can be isolated with the silt (50–2 μm) fractions (Tables 1, 2). Faunal activity may be important during these initial transformations[18, 29].

Table 1. Chemical analyses of organo-mineral size fraction from a native prairie soil (Blaine Lake, Black Chernozemic from Saskatchewan, Canada). Data compiled from Tiessen and Stewart[57], Tiessen *et al.*[39] and Tiessen *et al.*[38].

	mg/g fraction						
	C	N	P_o	Extractable P_o^1	$\delta_a^{15}N^2$ 0/∞	C/N	C/P_o
Whole soil	50	4.0	0.58	0.22	11	12.5	87
Sand (> 50 μm)[3]	27	1.9	0.19	0.06	7	14.0	141
Coarse silt (50–5 μm)	41	3.6	0.47	0.17	9	11.2	86
Fine silt (5–2 μm)	85	7.9	1.17	0.43	10	10.8	73
Coarse clay (2–0.2 μm)	82	8.7	1.67	0.56	12	9.5	51
Fine clay (< 0.2 μm)	63	8.0	1.16	0.37	17	7.9	54

[1] Organic P extractable in sodium bicarbonate and hydroxide
[2] $\delta_a^{15}N$ indicates the abundance of ^{15}N relative to air as parts per thousand excess
[3] The sand fraction also includes coarse, floatable (1.00 g cm^{-3}) organic matter

More labile portions of plant debris are consumed by the microbial population and will eventually enter the soil organic matter pool as microbial products. For instance, incubation of labelled ryegrass for several months showed that 85% of the C persisted in a light density fraction (2.00 g cm^{-3}, characteristic of partially decomposed litter) while 15% entered heavier organo-mineral complexes[28]. In contrast, a much shorter incubation with glucose, which rapidly passes into microbial decomposition pathways, resulted in 30% of the C entering the heavy fraction.

In a tracer experiment utilizing the naturally low abundance of ^{15}N in plant material, Tiessen and co-workers followed the sequential incorporation of prairie vegetation into the sand, coarse silt and fine silt fractions upon cultivation (Tables 1 and 2, 4 years). After four years large amounts of low abundance (plant derived) material were isolated in the colloidal fraction (< 0.2 μm) that is rich in microbial products. Continued cultivation for 60 years caused the rapid mineralization of the

Table 2. Changes in the chemical properties of organo-mineral size fractions of a Black Chernozem (Blaine Lake) with cultivation of 4 and 60 years. Data compiled from Tiessen and Stewart[37], Tiessen *et al.*[39], Tiessen *et al.*[38]

	% change from native				unit change		
	C	N	P_o	Extractable P_o	$\delta_a^{15}N$	C/N	C/P_o
4 years							
Whole soil	0	+6	+11	+7	−1	−1	−10
Sand	−43	−23	−26	−21	−2.5	−3	−40
Coarse silt	+28	+20	+28	+27	−2	0	0
Fine silt	+7	0	0	0	−1.5	0	0
Coarse clay	+7	0	0	0	0	0	0
Fine clay	0	0	0	+13	−6	0	0
60 years							
Whole soil	−34	−29	−20	−27	−1	−1	−15
Sand	−65	−68	−67	−71	+8	+2	0
Coarse silt	−26	−29	−28	−43	+3	0	0
Fine silt	−16	−27	−16	−40	0	+2	0
Coarse clay	−22	−28	−16	−29	−1	0	0
Fine clay	−46	−44	−32	−44	−7	0	−10

P_o = organic P
$\delta_a{}^{15}N$ = per mil ^{15}N enrichment compared with atmospheric ^{15}N abundance

recent organic matter of intermediate size, leaving the more stable fine silt and coarse clay associated organic materials behind (Tables 1 and 2) in conformity with data for Australian soils by Oades and Turchenek[30]. At this stage the effects of the original prairie biomass are entirely lost and the distribution of ^{15}N abundance across particle size fractions indicates a pattern of N cycling quite different from that of the native prairie. Simultaneously, available nutrients such as N and extractable organic P are substantially reduced (Table 2).

Organic matter stabilization

Both particulate and colloidal organic matter can undergo *physical stabilization* in the soil environment by association with many types of minerals. High $CaCO_3$ contents promote the stabilization of relatively undecomposed plant debris (inherited humin; Duchaufour[6]). Furthermore, resistant portions of organic substrates may be stabilized by mineral association before they can be incorporated into the soil biomass[19]. The colloidal fraction contains the microbial population and recent microbial products which can act as nucleation points for aggregate formation by binding clay particles and iron oxides[5, 8, 41].

Bacteria living in environments with large amounts of surface areas and high incidence of nutrient, temperature or moisture stress are

known to accumulate extracellular polysaccharides which may account for over 80% of the total biomass in activated sludge[14]. These polysaccharides have a high affinity for iron[4] and become resistant to decomposition due to precipitation reactions with metal ions or clay minerals[22]. Such organic matter–iron precipitates are a major component of soil humins[6], and may account for the greater stability of microbial products as compared to the original plant substrates[25]. Polysaccharides extracted from soils with alkaline reagents are important constituents of humic and fulvic acids. These acids hold cations very strongly against dialysis, acid treatment and substitution by Na during NaOH extraction, confirming their strong association in the soil[36].

Aggregates formed with polysaccharide cements in young pastures can initially be destroyed with periodate, while older ones are no longer susceptible to such treatment[11] suggesting progressive protection by mineral association. Organic matter–clay associations of intermediate density, formed in such a manner accumulate in pasture soils and break down under long term cultivation[30, 42].

The importance of organo-mineral associations (humins) as a sink for recent organic matter inputs was also shown by Goh *et al.*[10] who reported high levels of recent (bomb) ^{24}C in these fractions.

Particle size and density fractionations have pointed to the great importance of physical stabilization by mineral and metal ion interactions for the persistance of organic matter in soils. In addition, *chemical stability* may cause organic matter to accumulate. Aromatic compounds, in particular will enter into reactions leading to the formation of highly condensed resistant materials typical of the classical concepts of humid acid 'cores'[35].

Plant lignins are the primary sources of phenolic compounds in soil. Aromatic fungal metabolites are frequently plant derived but may be extensively modified from the original plant residues[23]. Phenolic compounds released into the soil are rapidly adsorbed to clays and amorphous Fe and Al minerals[15], where they may be catalytically polymerized into condensed ring structures as well as aliphatic polymers[26, 44]. Aromatic polymers, polysaccharides and other compounds containing N, S and P can co-polymerize[20], or simply coprecipitate with metal ions to form highly heterogeneous humic materials[23].

Addition of phenolic compounds to polysaccharide-mineral aggregates resulted in a considerable increase in aggregate stability[12], possibly due to a water-proofing effect. In this way normally transient, labile compounds were stabilized and preserved in the soil aggregate. The multiplicity of interactions between the different organic and inorganic components of the humic-mineral system makes small aggregates

(2–20 μm) very resistant to degradation under cultivation[41], and its constituent organo-mineral particle fractions show a very slow turnover even under prolonged cultivation[37].

Adsorption and precipitation reactions have been shown to occur with organic compounds at all stages of decomposition and chemical recalcitrance. The two modes of stabilization – physical and chemical[17] – are thus not exclusive but occur concurrently[33]. The resistant fraction of high radio carbon age (Gazineu, Anderson, Bettany, and Tiessen, Dept. of Soil Science, Univ. of Saskatchewan, unpublished results) that remains in the soil after long term cultivation and organic matter mineralization is therefore frequently little different in elemental composition (C:N:P ratio) from the younger more labile organic matter present in undisturbed prairie soils (Table 2).

Soil organic matter fractions and mechanistic models of turnover

Conceptual models in the past have emphasized the biological and chemical transformations and stabilization of soil organic matter. Physical processes such as sorption, precipitation and occlusion were recognized[17, 43], but for lack of adequate data were usually described as summary functions. This lack of data is explained by the inherent difficulty of extracting, isolating and analyzing physically protected organic matter. Data on its chemistry and structure have only recently been obtained on very few samples by *in situ* analysis using ^{13}C NMR[34] and by electron microscopical observation using specific stains on thin sections of soil fabrics[8].

Simple organic matter models that describe turnover as a function of the rates of transformation of the smallest possible number of organic matter pools under the influence of temperature and moisture do not require definition of the processes involved for their predictive purposes[32]. It may be necessary though to include soil mineralogy as a controlling variable in order to account for the widely differing kinds of organic matter stabilization in different soil types[6].

Models that are used as a framework for the conceptualising and testing of hypotheses on organic matter transformations[16, 43] will have to take account of the physical relationships of organic matter to its soil environment. Plant material enters the soil as particulates that can undergo biological/biochemical transformations or direct stabilization. Soluble litter components can be distributed through the soil matrix where they may be adsorbed to mineral surfaces, precipitated with other organic compounds or inorganic ions, or immobilized in the biomass. Since microbes are preferentially associated with stabilizing

surfaces, adsorption of organic compounds may only slightly retard their further turnover (*cf.* Filip[7]) while precipitation with cations, particularly iron, may cause prolonged stabilization[27].

Root exudates, which account for about 5% of the plant synthate[21], and microbial products enter the soil system as soluble or colloidal matter. Such materials frequently act as nucleation points for aggregate formation and sesquioxide precipitation, and physical stabilization may therefore be of primary importance. The study of this complex system of organo-mineral interactions in the future greatly depends on advances in *in situ* analysis utilizing ^{13}C, ^{15}N and ^{31}P NMR[45] as well as analytical electron microscopy[3, 9].

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A simple method for calculating decomposition and accumulation of 'young' soil organic matter

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Key words Accumulation Calculation method Decomposition rate Fertilizing system Organic manure Soil organic matter

Summary In previous simple models describing decomposition of organic matter, the rate of decomposition was usually assumed to be constant. Experimental evidence has shown this is not true. The objective of this study was therefore to find a relationship between decomposition rate and time. This resulted in an equation (Eqn 5), that can be used for many types of organic materials, provided their 'apparent initial age' is known. This age is related to the humification coefficient and varies from 1 year for green matter to 14 years for some peats. Formulas to calculate accumulation and decomposition of 'young' soil organic matter were also derived.

It was demonstrated that apparent differences in soil organic matter quality, found after 25 years of different fertilizing practices, were related to the proportion of young soil organic matter.

Introduction

Henin and Dupuis[3] were among the first to mathematically describe organic matter decomposition. They assumed that a fixed part of the organic material added to the soil is converted into humus. Kortleven[7] assumed this process of humification to be completed within one year. The fraction of the added material that was still present after one year, was termed the 'humification coefficient' **h**. He assumed the humified organic matter, would decompose at a constant rate **k**. The values of **h** and **k** were determined by comparing soil organic matter (SOM) contents in fallow fields and in neighbouring fields where roots and stubble had been ploughed in: the values were about 0.4 and 0.02 yr^{-1}, respectively.

In other studies the fate of organic material added to the soil was followed more directly[5,6,8]. Both, **h** and **k** were found to depend on the type of organic material used. For straw **h** was about 0.3–0.4, but **k** values of 0.10–0.15 yr^{-1} were found during the interval from 1 to 8 or 10 years after application, which is much higher than 0.02 yr^{-1}. Sauerbeck and Gonzalez[8] calculated that the equilibrium SOM content could be only one sixth of the actual SOM content, if **k** was 0.12 yr^{-1}. They

concluded that there must be two fairly independent organic matter pools in most soils: a smaller labile fraction consisting of plant residues and their 'humified' turnover products and a larger fraction of more stable organic matter.

It is clear that plant residues cannot have a constant decomposition rate. Initially there is a rapid breakdown of sugars, proteins and cellulose, the remaining material being more resistant. Also, the easily decomposable materials are partly resynthesized into microbial products which decay at an intermediate rate.

To quantify such processes Jenkinson and Rayner[4] , van Veen and Paul[9] and others developed complex simulation models.

Another approach was followed by Kolenbrander[5]. He considered the applied organic material as a whole and derived an equation indicating that decomposition rate decreases with time. The disadvantage was that for each type of organic material two constants were required. In this paper an alternative equation is presented, requiring knowledge of only one characteristic.

Relationship between k and time

If k is constant, it holds:

$$dy/dt = -ky, \tag{1}$$

where y = organic matter. Integration yields:

$$\ln y = \ln y_0 - kt \text{ or } y_t = y_0 e^{-kt} \tag{2}$$

where y_0 = amount of organic matter at t = 0. Therefore between t and Δt, **k** can be calculated by:

$$\mathbf{k} = \frac{1}{\Delta t} \ln \frac{y_t}{y_{t+\Delta t}} \tag{3}$$

The data of Kolenbrander[5,6] presented in Table 1 were used to calculate **k** values for subsequent time intervals. These **k** values gradually decreased with time, following similar patterns for each organic material. In a graph relating **k** to time, one curve could be fitted through all points: on log-log paper this resulted in a straight line (Fig. 1):

$$\log k = \log 2.82 - 1.6 \log t, \text{ or:}$$

$$k = 2.82\, t^{-1.6} \tag{4}$$

Equation 4 cannot be solved for t = 0. So a fixed 'starting time' was assigned to each type of organic material. This changes Eqn 4 into:

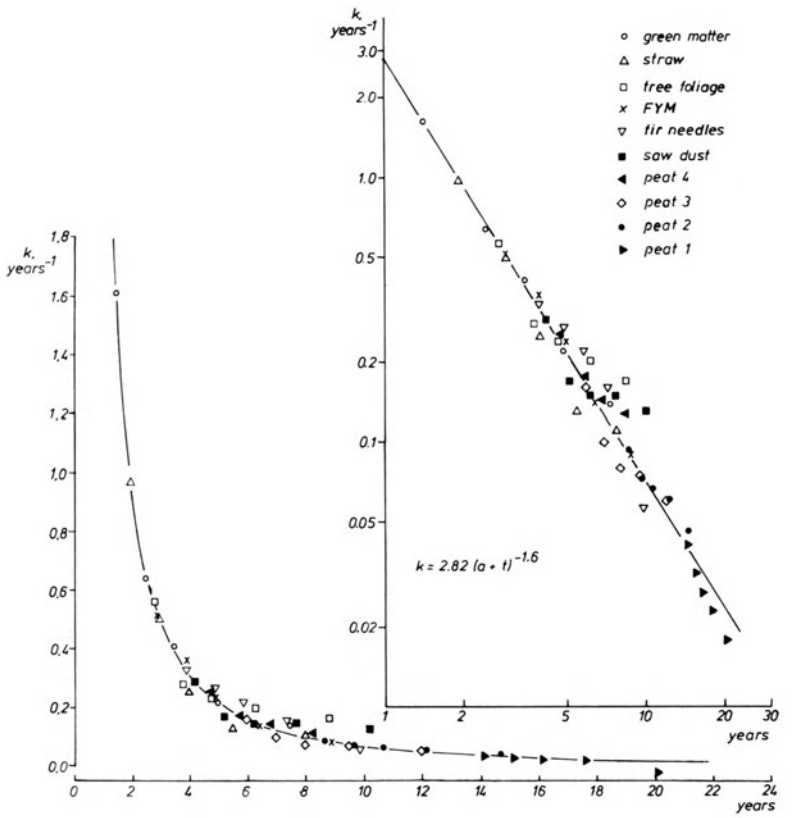

Fig. 1. Relationship between decomposition rate (k) and time (t) and between log k and log t for different organic materials.

$$k = 2.82\,(a + t)^{-1.6} \tag{5}$$

where **a** stands for 'apparent initial age' or shortly 'age'. Substitution of Eqn 5 into Eqn 1 gives:

$$dy/dt = -ky = -2.82\,(a + t)^{-1.6}\,y \tag{6}$$

Integration yields:

$$\ln y = \frac{-2.82}{-0.6}\,(a + t)^{-0.6} + C \tag{7}$$

If $t = 0$, $y = y_0 = X$, being the amount of added organic matter. So the integration constant $C = \ln X - 4.7\,a^{-0.6}$. Substitution of C in Eqn 7 and rearranging gives:

$$y_t = X \exp 4.7\,\{(a + t)^{-0.6} - a^{-0.6}\} \tag{8}$$

Table 1. Remaining organic matter (%) after addition to soil of different organic materials (after Kolenbrander[5,6]) and apparent initial age (a), as calculated with Eqn 8

Organic material	Years since application 0	1	2	3	5	8	a (yr)
Green matter	100	20	10.5	7	4.5	3	0.99
Straw	100	38	23	18	14	10	1.41
Litter	100	57	43	34	23	14	2.28
Farmyard manure	100	60	42	33	25	19	2.45
Fir needles	100	72	55	44	32	27	3.36
Sawdust	100	75	63	54	40	27	3.69
Peat 4	100	77	64	55	43	n.d.	3.95
Peat 3	100	85	77	71	61	51	5.47
Peat 2	100	91	84.5	79	70	61	7.87
Peat 1	100	96	93	90.5	86.5	82	13.62

If any y_t is known, the value for a can be found with Eqn 8. In practice, this usually comes down to knowledge of y_1, *i.e.* of the humification coefficient, $h = y_1/X$.

The values of **a** are given in Table 1 and are assumed to be valid only for temperate climates.

In cases where sufficient empirical data are available, it might be better to use a more general equation instead of Eqn 5, in the form of:

$$k = -p(a + t)^{-q} \tag{9}$$

and to solve p, q and **a** by fitting the data.

Accumulation and decomposition of 'young' soil organic matter

By *young* SOM is meant that part the SOM which has been accumulated by repeated additions of crop residues and organic manure since $t = 0$, being the start of a certain treatment or trial. It can be calculated by summation of subsequent y_i's:

$$\sum_{i=0}^{t} y_i = y_1 + y_2 + y_3 + \ldots + y_t \tag{10}$$

Table 2 gives an example for farmyard manure (FYM). Eqn 10 implies that added organic matter is not considered as young SOM, until it has been in the soil for at least one year.

The decomposition of young SOM during a certain time interval can be calculated by summation of the decomposition of the subsequent y's. Thus, decomposition during the fourth year (Table 2) is: $(35 - 30) + (44 - 35) + (60 - 44) = 30$. A shortcut is: $139 + 60 - 169 = 30$.

In situations where every year the same amount and type of organic matter is applied to the soil it would be desirable to replace Eqn 10 by

Table 2. Accumulation of young soil organic matter, as calculated for annual additions of 100 units of FYM (rounded figures). Humification coefficient **h** = 0.60 and **a** = 2.45 years

Number of application	Years after first application 1	2	3	4	5	6	7
1	60	44	35	30	26	24	22
2		60	44	35	30	26	24
3			60	44	35	30	26
4				60	44	35	30
5					60	44	35
6						60	44
7							60
Total	60	104	139	169	195	219	241

Table 3. Some data pertaining to the 'Thee Organic-Matter-Farms' soils (0–25 cm), as analysed by van Dijk[1] and as calculated with the proposed model

Farm	I	II	III
Analysed			
bulk density, kg dm^{-3}	1.50	1.45	1.35
total SOM, tons ha^{-1}	78.4	81.9	86.9
total N, tons ha^{-1}	4.01	4.35	4.56
C/N	11.3	10.9	11.0
N mineralisation (6 weeks incubation),			
mg N per kg soil	14	17	25
mg N per g total N	13.1	14.2	18.5
Calculated			
young SOM, tons ha^{-1}	6.2	11.0	15.2
old SOM, tons ha^{-1}	72.2	70.9	71.7
decomposed *young* SOM, kg ha^{-1} yr^{-1}	660	1382	2049
N mineralisation,			
from young SOM kg ha^{-1} yr^{-1}	34	74	108
from old SOM kg ha^{-1} yr^{-1}	26	26	26
total kg ha^{-1} yr^{-1}	60	100	134
mg N/kg total N yr^{-1}	15.0	23.0	29.4

an integration formula but this is mathematically impossible. The advantage of Eqn 10 is that it can be used in practical situations where organic material additions change from year to year in quantity, quality and time of application. The utility of the proposed calculation model is illustrated by the following example.

A practical application

During the period 1951–1976 three fertilizing systems were compared on the so-called 'Three Organic-Matter Farms' in the North-East Polder, the Netherlands:

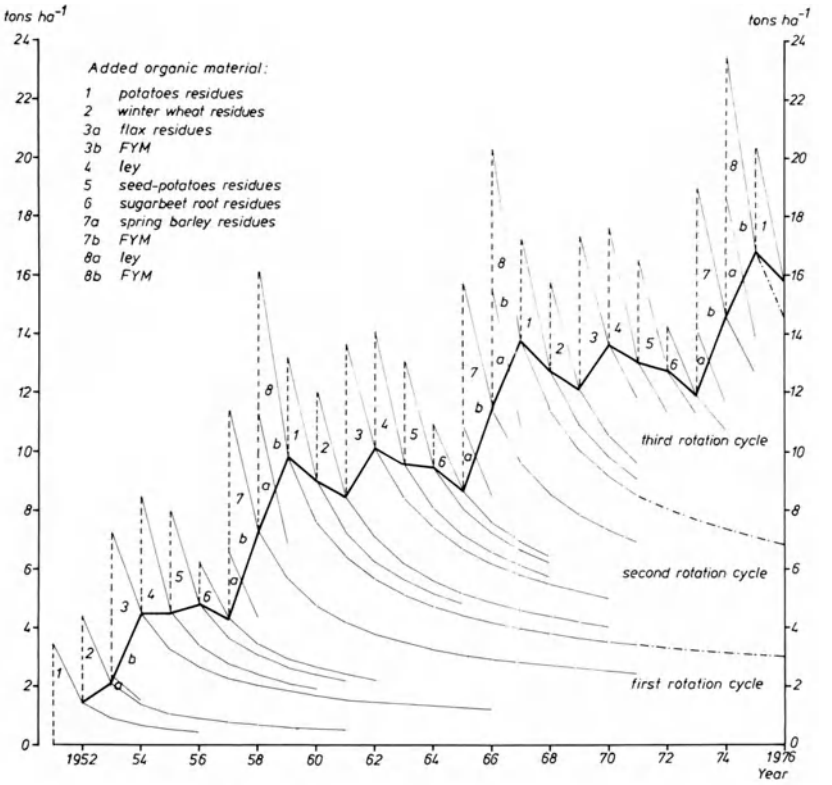

Fig. 2. Accumulation of young soil organic matter on the 1976 wheat plot of Farm III during 1951–1976.

I mineral fertilizers only
II mineral fertilizers and green manures
III mineral fertilizers, ley and farmyard manure

Systems I and II had a six year rotation of potatoes, winter wheat, flax, seed-potatoes, sugar-beet and spring barley. Green manures in system II were clovers, ryegrass and sugarbeet tops and leaves. In system III flax and spring barley were followed by a one year ley, while FYM was applied three times during an eight year cycle. Farms I and II had six and Farm III eight plots, hence all crops of a rotation cycle could be grown yearly[2].

In 1976 samples were taken from the two plots where winter wheat and then sugarbeet were grown once in 8 years. Table 3 gives the mean values of some analytical data from van Dijk[1]. A striking feature was that N mineralization was not proportional to organic N content. Similar trends were found for dehydrogenase- and phosphatase activities

and for the amounts of mineral N present in the profile in March 1976. Apparently different types of SOM had developed under the different fertilizing systems.

It was hypothesized that the difference was related to *young* SOM accumulated since 1951. The amounts of crop residues, green and farmyard manure and their humification coefficients were estimated as accurately as possible and next *young* SOM was calculated with the aid of Eqn 10. Fig. 2 illustrates data for the plot of Farm III where wheat was grown in 1976: the vertical dashed lines indicate the yearly added fresh organic matter and the thin solid lines what remained subsequently and the thick line connects the totals of the young residues that are at least one year in the soil. Clearly FYM made the most important contribution to this *young* SOM (years 3, 7, 8). In the other years the newly added *young* SOM could not compensate for the losses by decomposition of the previous *young* SOM.

The lower half of Table 3 presents some calculated data, again averaged for the wheat and sugarbeet plots of 1976. The values for *old* SOM were calculated by substracting *young* SOM from total SOM. *Old* SOM is about equal for the three farms, which gives confidence in the followed calculation procedure.

N mineralization was calculated as: 0.58 x SOM decomposition/(C/N). Decomposition rate of *old* SOM was assessed at $0.007\,yr^{-1}$ (details given in internal documents). The calculated N mineralization increases in the order farm I – II – III, which is the same sequence as for the measured N mineralization.

So, the observed difference in SOM quality between the farms arises principally from differing proportions of *young* and *old* SOM. As *young* SOM decays rapidly, soils with much *young* SOM exhibit high relative N mineralization rates. It must be admitted that this example refers to very uniform soils but, under less ideal conditions, Verveda[10] found that N uptake by field crops receiving no fertilizer N was related to the calculated amounts of *young* SOM.

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Section 6

Interaction effects of organisms, organic matter and management on soil structure

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Interactions between biological processes, cultivation and soil structure

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Key words Autochthonous Biomass Cultivation Inoculation Lignocelluloses Polysaccharides Rhizosphere Soil stability Soil structure Zymogenous

Summary An inherent (autochthonous) biomass is characteristic of a soil while the input of substrates for plant roots or crop residues promotes the transient (zymogenous) biomass. However successful micro-organisms will show aspects of both types of ecological strategy. The biomass generated from plant residue substrates can include toxin-producing and pathogenic species but also beneficial organisms such as N-fixers and polysaccharide-producers. Rhizosphere activity can, depending on soil, plant and microbial species, stabilize or destabilize soils. Microbial activity should be considered in soil management and it may be possible to manipulate the soil population balance towards beneficial organisms.

Introduction

During the long history of cultivation, a fundamental objective of agricultural science has been to assess the effect on the soil. Evaluations at both the advisory and research level are usually conducted by teams of agronomists, plant physiologists, plant pathologists, soil chemists and soil physicists. It is relatively rare to find soil biologists in such teams and this may be because in the past they have had little to contribute. The science of soil microbiology has tended to be a process-oriented discipline with a strong emphasis on concepts. There has been a tendency to either count the number of viable cells which can be isolated on a selective medium or to isolate individual organisms from the field and demonstrate their biochemical potentials in defined nutrient-rich conditions in the laboratory, without evaluating the potential of the micro-organism back in the agricultural system. To an extent, the biologist can be excused in that suitable techniques were not readily available. However, as we now have a new armoury of methods, for example to determine soil biomass (the total living material in soil excluding roots), the challenge is on.

When evaluating activities in the field, it is vital to consider the underlying fundamental principles and concepts for study. The most obvious but important of these is, perhaps, that there will be no growth

of heterotrophic organisms in soil unless a suitable C and energy substrate is available. Generally, the combined soil organic matter (humus) which usually accounts for by far the greatest fraction of soil C, does not provide a suitable substrate because it has a half-life in excess of 1000 years. However, if the soil is sterilized or air-dried and re-wetted, sufficient C is released from the breakdown of the humus or killed cells to account for the flush in respiration[4] or microbial proliferation[37] that will take place. The principle limitation on growth in soil appears to be substrate availability and not the presence of toxic substances[32,37]. Lack of microbial proliferation in soil is often termed 'microbiostasis' but it seems to me preferable to specify lack of growth or toxicity. The two principle sources of C to the biomass are roots (living or dead) and shoots (falling leaves or crop residues).

The manner in which micro-organisms acquire available substrates in soil was first considered by Winogradsky[61]. The *zymogenous* or fermentative organisms rapidly increase in biomass in response to the input of fresh substrates, but they die out rapidly unless their energy demands are met. By contrast, the soil also supports a relatively constant biomass, which responds little to the input of fresh substrates; this is the *autochthonous* population which needs only a small supply of energy for its survival. In modern ecological terminology, the former group can be considered as r strategists and the latter as K strategists[5]. In practice it is probably unwise to consider these distinctions too rigidly as the completely successful soil organism will certainly exhibit characteristics of both types of ecological adaptation.

The soil biomass and cultivation systems

In the present essay, micro-organisms will receive greatest attention because they usually constitute the major fraction of the soil biomass.

Classical approaches to biomass determination were to assess the biovolume of cells by direct observation. This is both tedious and unreliable, even with modern fluorescent staining techniques[23]. A major breakthrough came when Jenkinson and Powlson[26] showed that by fumigating soil with the chloroform, the rate of mineralization from the dead cells to CO_2 was proportional to the biomass originally present, as assessed by biovolume. However, there may be a fallacy in that the technique can never be regarded as absolute when the calibration method was unreliable. A range of other methods followed, such as a modified fumigation technique where glucose was added to soil and respiration measured over a short (5 h) period[2], P release following fumigation[6], ATP determination[28] and microcalorimetry[57]. In comparative surveys[23,57],

it seemed that the original fumigation method[26] was generally most reliable, although it appeared less useful for acid soils. A problem with all the methods as described is that they depend on the use of sieved soils and such action can be regarded as more stringent than the passage of a plough through the soil. Lynch and Panting[44,47] found for samples taken in winter than sieving resulted in a smaller biomass yield than that obtained in intact cores, possibly because saprophytes dominated and the sieve removed substrates and fungal cells. In summer, the rhizosphere contributed much more to the total soil biomass and the increased biomass, measured on sieving may be the result of activation of the bacterial cells. In spring samples there was no observed effect of sieving so these effects may cancel out[27,47].

The fumigation-respiration technique with intact cores has been particularly suitable for measuring effects of cultivation; it does not depend on an absolute determination but rather a comparison between control and treated soils. This has been useful in demonstrating the seasonal variation in biomass[44,45]. As roots grow, the soil biomass increases by utilizing the C output from the roots so that at the time of maximal root production, biomass is greatest. In direct-drilled crops, root proliferation in the surface layers (5 cm deep) of soil is often greater than that from crops drilled after ploughing; accordingly, biomass is then greater in these soils. When sampling to greater depths (25 cm), the biomass was greater in direct drilled compared with ploughed soils at only one of four sites[53]. This is not particularly surprising because if root production is a major factor determining biomass, the deeper sampling will tend to provide an integrated figure for the biomass down the profile. Thus tillage may not change the overall soil biological activity, but it probably alters its distribution in the profile.

Grassland soils contain a greater root biomass than arable soils by virtue of the perennial nature of the crop. Consequently, the microbial biomass of grassland soils is usually at least double that of arable soils[1,27,45].

Burning of straw residues removes a major portion of the available substrate into microorganisms in soil and thus the biomass can be greater in the surface layer if straw is left on the surface[45]. However, straw is utilized by soil microorganisms more slowly than root exudates for example. Though there may be a transient increase in biomass during the early stages of rapid straw decomposition, subsequent increases are small and difficult to detect[47].

When N fertilizer was applied in spring, the biomass in both March and July increased where the soil had been direct-drilled, but not where

it had been ploughed[47]. The increase in biomass suggests that a fraction of the soil biomass is N-limited, but the precise interpretation is uncertain. The extra biomass following fertilizer additions was on average $100\,kg\,C\,ha^{-1}$ in the surface 5 cm. If the C:N ratio of microbial cells is assumed to be about 5:1, this means that about $20\,kg\,N\,ha^{-1}$ is immobilized in the microbial biomass. This is similar to the extra $25\,kg\,N\,ha^{-1}$ that is often recommended should be added to the seedbed when direct drilling (MAFF 1979)[50]. If that N were in the autochthonous biomass, its release would take much longer than if it were in the zymogenous population. Little is known about the recycling of N from biomass, but such information is desirable from both basic and practical standpoints.

The use of slurries and farmyard manures in agriculture is somewhat similar to the incorporation of straw into soil in that a complex substrate becomes available to microorganisms only relatively slowly. In an experiment initiated in 1893 at the Askov Experimental Station in Denmark, the biomass in unmanured, manured and NPK fertilized plots was recently compared[12]. Microbiological activity was compared by plate counts of bacteria and fungi, ATP content, oxygen uptake, dehydrogenase activity and biomass determined by fumigation. In general the differences were small, these observations being confirmed in a subsequent experiment initiated in 1972[13]. Thus again, variations in the size of the background or autochthonous biomass in the soil appear to be buffered against an agricultural practice which is providing a large substrate input to the soil.

Changing agricultural practices, such as direct drilling have resulted in increased herbicide and pesticide usage. It is critical to assess the effects on the soil biomass of such chemical inputs at rates used in the field. Anderson *et al.*[3] found that at $5\,\mu g\,g^{-1}$ soil the fungicides captan, thiram and verdasan caused transient decreases of 40% in the biomass and a shift in the soil population balance from fungi to bacteria. Long-term decreases were observed when the fungicides were applied at $50\,\mu g\,g^{-1}$. Thus although effective field application rate is difficult to repeat in laboratory experiments biomass determinations are a useful monitor of the environmental input of agrochemicals and it seems pertinent to measure biomass in field plots.

Problems and benefits of plant residues

In Britain, unlike most countries, straw from one crop is usually burnt prior to drilling a succeeding crop. Although some consider this environmentally unacceptable, there is a clear agronomic advantage

because if a crop is direct drilled beneath unburnt straw in a wet autumn, crop yield is reduced by about 20%[42]. The problems are lessened if the straw is ploughed in[15]. Mechanical difficulties in drilling can be a cause of the problems but even if they are eliminated, biological problems remain[39]. Straw, which is a lignocellulose, provides a potential substrate to pathogenic and other harmful microorganisms. In practice, there is little evidence for a build-up in pathogens under such situations, except one report where *Pythium* infection increased[9]. There is some evidence for apparently benign pseudomonads entering the root cortex as a result of the presence of straw in soil; these can stunt root growth, an effect which depends on the plant cultivar[14]. However, a major interpretation of the adverse effect in that the cellulosic fractions of straw provide a substrate to fermentative bacteria under the wet soil conditions and yield phytotoxic concentrations of acetic and other organic acids[35,58]. This effect has been demonstrated in many laboratory and field investigations and is not restricted to straw. Grass and weed residues can cause similar problems, although in these cases the build-up of pathogenic fungi, particularly *Fusarium culmorum*, is probably relatively more important[21,52].

Straw has a large C:N ratio (*c.* 100:1) whereas the decomposer microorganisms have a much smaller ratio (*c.* 5:1). Thus soil or fertilizer N tends to be immobilized into biomass during the microbial decomposition of straw. Such immobilization is however only temporary. We know too little about the kinetics of recycling of N contained in microbial biomass although the question has been addressed by Ladd[31]. My studies with S H T Harper[38] indicates that the N can be released from biomass during the spring, when the crop is most in need of it. The immobilization would in that case be advantageous because it would tend to prevent winter leaching. There is clearly a need for ^{15}N studies to evaluate such potentials.

Whereas C inputs can increase denitrification in soil, particularly under wet conditions, there is also potential for N-fixing populations to be stimulated. Studies with mixed populations of cellulolytic and N-fixing organisms utilizing cellulosic substrates have shown N gains of 6–14 mg/g substrate consumed[29,43,56]. The fixed N appears to be available to plants[25] at about the same rate as fertilizer N. A cereal crop yielding 7 t straw ha^{-1} treated in this way to provide 5 mg N/g straw, if scaled-up to a simple farm-scale operation, would provide the equivalent of 35 kg N ha^{-1}. As the straw already contains about 3 mg N g^{-1}, the total N content of the straw would amount to 57 kg N ha^{-1}, or about half of the annual fertilizer application to arable crops.

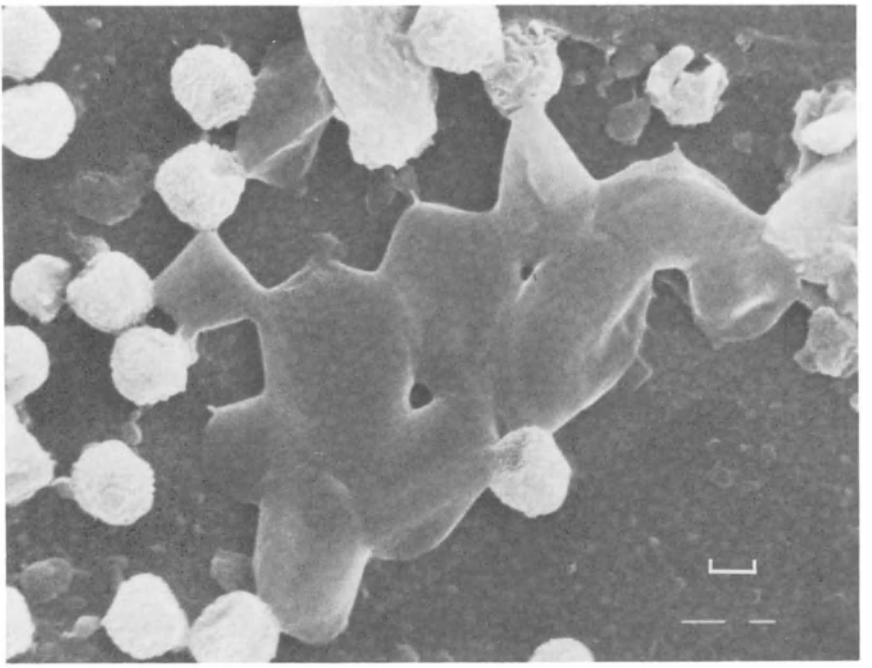

Fig. 1. Bacteria on the surface of straw producing a gum which stabilizes soil aggregates. Bar marker 1 μm. (From collaborative work with Dr L F Elliott, USDA, Washington State University.)

One of the major reasons for not burning straw in the Pacific Northwest of the United States is that it is retained as part of a conservation tillage system, reducing water and wind erosion[40]. Clearly there is a physical involvement but there is also a biochemical effect where microorganisms produce extracellular polymers, particularly polysaccharides, which provide a cementing action between soil particles, and fungal hyphae bind around the aggregate. This produces aggregates which are stable to the action of water[22,24,49,60]. Plant residues provide energy to 'fuel' the biomass for this function[20,48], which is particularly critical where soils are subject to instability problems. A classical example of unstable soil is volcanic ash. Following the eruption of Mt. St. Helens, Washington State received a covering of ash which had a particle size composition equivalent to the silt loam soil of the region. Laboratory experiments with this 'organic matter-free soil' demonstrated that microbial degradation of straw (Fig. 1) yielded aggregating agents which produced water stable aggregates of the ash and the soil of the region[40]; undegraded straw had no significant effect on stability. Subsequent

experiments (L F Elliott, J M Lynch unpublished) with straw containing various quantities of available C and N showed that straw with the smallest N content (0.25% w/w) resulted in significantly greater aggregation of the soil and ash than the larger N content straws (up to 1.09% w/w).

Water-soluble polysaccharides were extracted from wheat straw composted in glass columns (S J Chapman, J M Lynch unpublished). The neutral sugar content showed the polysaccharide to be of microbial origin but with some hemicellulose-like material when compared with the sugars presented in the fresh straw.

Rhizosphere activities and soil structure

As already indicated, the growth of plant roots in the soil increases the microbial biomass. In general, the stability of soil aggregates increases with biomass (Lynch[36]; E Henness, J M Lynch, M Fletcher unpublished), although the relationship between biomass and stabilizing effect depends on the microbial species (Table 1). However, in an unstable sandy soil, an increase in the biomass of the fungus *Mucor hiemalis* in soil caused a decrease in aggregate stability[36]. It is recognized in practice that the introduction of a grass (or lucerne) ley into arable cropping is a means of improving soil structure but the number of scientific investigations of such effects are small[8,10,33]. The deleterious effect of arable cropping on soil structural condition is also recognized[34,51].

The influence of root growth on soil aggregate stability (measured by turbidimetric and wet sieving procedures) was investigated using five crop species and two soils contained in pots[54]. Aggregate stability of soil contained in pots was improved by the growth in the soil of perennial ryegrass and lucerne for 42 days, whether tested in the fresh or air-dried state. Periodate-sensitive (probably polysaccharides) materials were involved. By contrast, the growth of maize, tomato and wheat for

Table 1. Effect of added bacteria (*Pseudomonas* spp) on the aggregate stability of an irradiated clay loam soil (*Denchworth series*). Data provided by Miss E Henness

Bacterium	Regression line[+]	Correlation coefficient
G1	$y = 0.220x + 0.650$	0.84^{**}
G9	$y = 0.111x + 0.696$	0.79^{*}
G13	$y = 0.026x + 0.632$	0.96^{**}
G15	$y = 0.109x + 0.656$	0.90^{**}

[+] y is the stability index (ratio of transmittance of sample to transmittance of water) as assessed by the turbidimetric assay and x is the dry weight of bacterial cells added (mg g^{-1} soil).

25 days could cause a decrease in the stability of fresh soil aggregates, even though this was not apparent after air-drying when periodate-sensitive materials were released as a consequence of the drying. No attempt was made to distinguish between the effects of the roots *per se* and the associated microbial biomass.

The destabilizing effect of the growth of maize roots in the soil was investigated by treating the soil prior to determining aggregate stability with sodium periodate to assess the importance of polysaccharides, and acetylacetone to assess the importance of organically bound Fe and Al prior to determining aggregate stability[55]. This suggested that the destruction of (organic matter)–(Fe or Al)–(mineral particle) linkages largely accounted for the effect and it seemed that removal of the Fe and Al cations by chelating agents released in the rhizosphere was the most likely mechanism. However, recent studies in my laboratory (E Henness unpublished observations) have indicated that the destabilizing effect of maize root growth is not general.

In studies of the effects of roots on soil aggregate stability, few attempts have been made to distinguish between the effects of the roots *per se* and that of the associated microbial biomass. Tisdall and Oades[59] suggested that vesicular-arbuscular mycorrhizal fungi (VAM) were responsible for the increase in aggregate stability associated with the root growth of ryegrass. They concluded from direct measurement of hyphal lengths and scanning electron micrograph (SEM) pictures, that the greater stabilizing effect of ryegrass compared to white clover roots was due to the larger population of VAM present in the soil.

Forster[18] provided microscopic evidence that fungi aggregated sand is an embryo dune system and later observations[19] showed that fungi (*Glomus fasciculatus* and *Penicillium* sp) were only really effective in aggregating the sand when roots of the pioneer grass (*Agropyron junceiforme*) were present. Reid and Goss[54] did not find a correlation between the VAM population on roots and soil aggregate stability. It seems likely therefore that in some situations, symbiotic growth of VAM fungi on plant roots will lead to improvements in aggregate stability of soils, and sometimes the growth of saprophytic fungi on root-derived C will be responsible. However neither situation is necessarily the general case and there appears to have been no studies where the relative effect of rhizosphere bacteria has been quantified. Faull and Campbell[16] provided excellent pictorial evidence by transmission electron microscopy (TEM) that a rhizosphere colonist of wheat, *Bacillus cereus* var *mycoides*, was surrounded by an electron transparent region, presumably polysaccharide, to which clay particles

adhered. Subsequently the significance of attachment of clays to bacteria in solution culture was studied in relation to its potential for control of fungal disease[7].

In a simple model of aggregation, Fletcher *et al.*[17] showed that bacteria and clay particles in the fine pores of the aggregate provided a 'cementing' action with the biopolymers produced. Charge interactions were provided by polarization of the net negative charge on the clay and bacterial surfaces or by metal ion bridging between the two. Tisdall and Oades[60] classified aggregate organization according to the size of aggregate: roots and hyphae (200 μm), plant and fungal debris encrusted with inorganics (20 μm), microbial and fungal debris encrusted with inorganics (2 μm) and amorphous aluminosilicates, oxides and organic polymers sorbed on clay surfaces and electrostatic bonding with flocculation (0.2 μm). Such conceptual schemes have been supported to an extent by the excellent TEM pictures of Kilbertus[30]: he was able to quantify the presence of open and closed pores within an aggregate by taking sections across it. Chemically the organic binding agents can be classified as transient (mainly polysaccharides), temporary (roots and fungal hyphae), and persistent (resistant aromatic components associated with polyvalent metal cations, and strongly sorbed polymers)[60].

Conclusion: Soil management

It might be argued that turning the soil with a plough, providing aeration and making otherwise inaccessible substrates available for microbial metabolism would promote degradation of the soil organic matter, including aggregating agents, and therefore result in a decline in soil structure. When sampling to just below plough depth, Powlson and Jenkinson[53] found no change in soil organic matter as a result of cultivation. However, in the surface (2.5 cm deep) aggregates of a range of soils, Douglas and Goss[11] found that both organic matter content and aggregate stability generally decreased with tillage and could be enhanced by direct drilling, an Andover series silt loam being an exception. It is therefore dangerous to generalize on the effects of cultivation on all soils in all horizons. The improvement in stability measured as a result of the introduction of grassland[11] does appear to be a general phenomenon but other crops vary in their effects.

The relative quantitative roles of roots and microorganisms in the processes are still uncertain; very little attention has been addressed to the role of earthworms and their gut microflora in soil aggregate stabilization during the utilization of plant residues. However the question arises as to whether the natural roles of microorganisms may

be manipulated by inoculation of suitable aggregate-forming micro-organisms. Clearly the use of microbial cells *per se* would be inappropriate, except perhaps algae for the soil surface where there might be a continuous source of light and mosture. There would be more scope if the inoculum were produced on plant waste, such as straw, and incorporated into soil after a suitable period of decomposition[40]. However, even with very unstable soils, the procedure might only be economic if the inoculum provided other useful functions such as the minimization of straw toxicity[41] and pathogen colonization, or the provision of plant nutrients, particularly N[43]. Such approaches should be considered as useful targets for soil biotechnology[38].

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Soil organic matter and structural stability: mechanisms and implications for management

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Key words Aggregates Aluminium Bacterial mucilage Binding agents Calcium Cation bridges Complexing agents Dispersion Electron microscopy Electrophoretic mobility Fungal hyphae Glues Iron Management Periodate Polysaccharides Rhizosphere Roots Slaking

Summary The stability of pores and particles is essential for optimum growth of plants. Two categories of aggregates macro- (> 250 μm) and micro- (< 250 μm) depend on organic matter for stability against disruptive forces caused by rapid wetting. Dispersion of clay particles from microaggregates is promoted by adsorption of complexing organic acids which increase the negative charge on clays. The acids are produced by plants, bacteria and fungi. However, the dispersibility of clay in microaggregates is offset by the binding action of polysaccharides, mainly mucilages produced by bacteria, but also by plant roots and fungal hyphae. The stability of microaggregates is also enhanced by multivalent cations which act as bridges between organic colloids and clays. Macroaggregates are enmeshed by plant roots, both living and decomposing, and are thus sensitive to management, and increase in number when grasses are grown and the soil is not disturbed. Lack of root growth, *i.e.* fallow, has the opposite effect. Various implications for management of soil structure are discussed.

Introduction

Soil structure is defined as the size and arrangement of particles and pores in soils. Good structure for plant growth on loams and clays can be defined in terms of the presence of pores for the storage of water available to plants, pores for the transmission of water and air, and pores in which roots can grow (Table 1). The particle sizes associated with such pores are indicated so that we can relate pore and particle diameters. A desirable range of pore sizes for a tilled layer occurs when most of the clay fraction is flocculated into microaggregates, defined as $< 250\,\mu m$ diameter, and secondly these microaggregates and other particles are bound together into macroaggregates $> 250\,\mu m$ diameter. The majority of the macroaggregates should have diameters in the range 1 to 10 mm[6, 21]

The architectural arrangement described, whether natural or created by tillage, must be stable to disruption on wetting. The breakdown of macroaggregates on wetting due to entrapped air and unequal swelling

Table 1. Functional classification of soil pores and associated particles

Pore diameters (μm)	Function	Particle diameters (μm)
< 0.2	Bound water	< 2
0.2–2.5	Storage of water available to plants	2–250
25–100	Capillary conduction, aeration	250–1,000
> 100	Aeration, fast drainage root growth	> 1,000

has been termed slaking[7]. Slaking results in microaggregates from which clay particles may or may not be detached (dispersed) depending on factors described in double layer theory, and some that are not including particle size, shape, packing and the input of energy. Slaking alone is a problem and causes reduction in infiltration rate of rainfall or irrigation water, and in hydraulic conductivity. When dispersible clay is also present porosity is decreased, soil strength increased and generally unfavourable physical conditions prevail[41, 42].

Organic materials in soils influence both dispersion–flocculation phenomena and the stability of macroaggregates.

Microaggregates

Swelling and dispersion

Organic anions which form complexes with metal ions favour the dispersion of clays in a manner similar to that of Calgon and other phosphates. At least two factors are involved: a) the complexing of trivalent and divalent metal ions which reduces concentrations in solution, and a reaction with any positive sites associated with trivalent metals situated on the edges of clay lattices or on oxide surfaces, and b) the sorbed organic anions increase the negative charge on colloid surfaces thus increasing the diffuse layer of cations associated with the surfaces, favouring the dispersion of clay particles.

The influence of organic matter on the dispersion of clay has been illustrated convincingly by Gillman[12] in soils developed from basalt. Weathering of the ferromagnesian minerals has produced a soil clay rich in oxides. At depth this clay dispersed as a colloid with a net positive charge, *i.e.* the point of zero charge (PZC) of the clay was higher than the pH of the soil. Closer to the soil surface where the organic matter content was greater the PZC matched the pH of the soil and there was no water dispersible clay (Fig. 1). Within about 30 cm of the surface of the soil substantial quantities of water dispersible clay were present as the PZC was lowered below the pH of the soil by the absorption of organic materials.

The adsorbed organic materials must have been largely anionic in

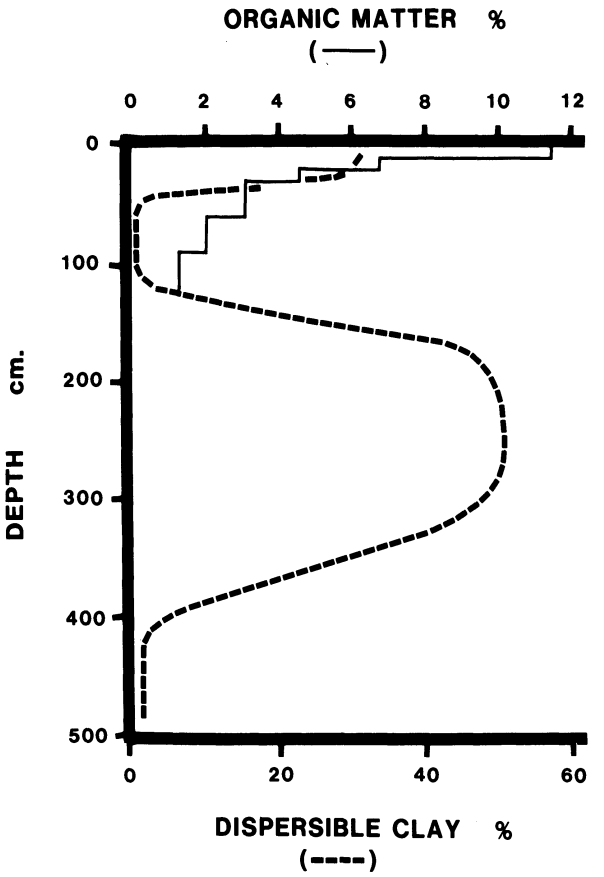

Fig. 1. Organic matter content and dispersible clay (from Gillman[12]).

character. It has been shown that sorption of a range of organic anions on a soil clay to which Fe polycations had been added increased the dispersibility of the clay due to changes in the surface properties[43]. Polycations of Fe were added to a soil containing 18% clay, as determined by standard procedures, until the PZC coincided with the pH of the soil when all the clay was flocculated. To this clay a range of anions was added and the amounts of dispersible clay were determined (Fig. 2).

The anions studied were divided into four groups on the basis of their influence on dispersible clay. The effect of phosphate was predictable based on the use of various phosphates to stabilize dispersions of soil clays in the laboratory and in farm dams. The efficiency of the fulvic acid preparation was surprising because Fe-fulvate has a low stability constant. However, fulvic acid is strongly sorbed on iron oxide surfaces as carboxyl groups replace hydroxyl groups giving multiple points of contact[36]. The relative efficiencies of the other anions was

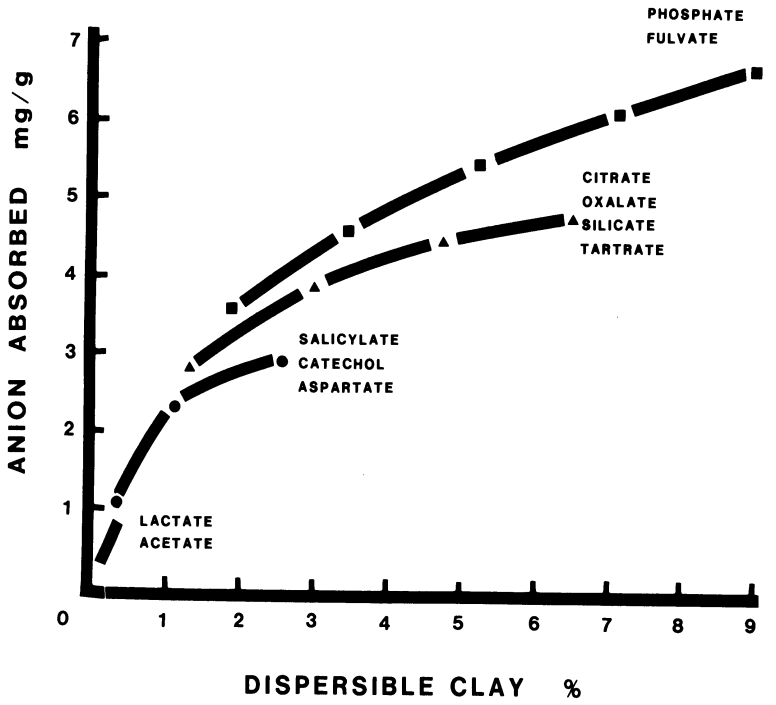

Fig. 2. The dispersion of clay by adsorption of organic anions.

related to the energy of binding of the anions to the Fe on the clay surface.

The anions clearly increased the negative charge on the clay particles as shown by the substantial negative electrophoretic mobilities for the two groups of anions adsorbed in greatest quantity, which were also those absorbed most strongly (Fig. 3). The increased dispersibility of the clay caused by the adsorption of the anions increased the bulk density of the soil and decreased the water holding capacity. Simultaneously the modulus of rupture was increased and the friability decreased. It would be informative to repeat this experiment in a system where positive sites originated from Al rather than Fe.

The range of acids produced in soils has been described by Stevenson[45]. Citric, oxalic and tartaric acids are produced by plants, bacteria and fungi. The role of these acids, and a wide range of others, in the process of podzolization has been described by many authors (see Petersen[37]). The emphasis has been on the dissolution and complexing of metals as a means of translocation of Al and Fe. However, it is likely that acids, from simple well defined compounds through to the fulvic acids, are involved in dispersion of clays in surface horizons and clay transport to lower horizons (illuviation, lessivage). This dispersion of clay will be important in soils with a pH of 6 ± which are leached free

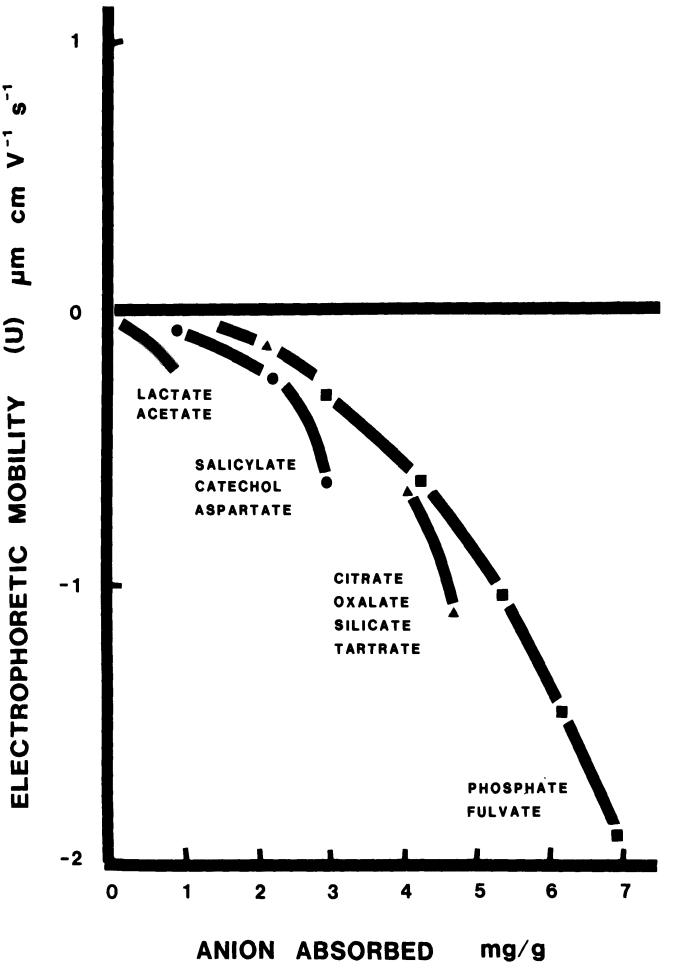

Fig. 3. The influence of anion adsorption on the electrophoretic mobility of a soil clay.

of $CaCO_3$ but are not sufficiently acid for concentrations of soluble Al to be important. The soils do not need to be sodic. A range of duplex soils occupy 20 per cent of the Australian continent and many are notorious for poor physical conditions, associated with dispersible clay, which lead to surface crusts and 'hard setting' properties.

Because roots and rhizosphere organisms produce a range of acids one might predict that the living root system organisms would stimulate production of dispersible clay. Such an effect has been described by Reid and Goss[39, 39] and Reid *et al.*[40], particularly for maize plants during the early weeks of growth. The increased dispersion of soils after growth of maize plants for 14 to 42 days was evident if soils were maintained moist prior to analysis. The acids produced could break

organic matter – Fe. Al. Ca-clay bonds by lowering the pH and/or complexing the metal ions. Iron may also be dissolved in the rhizosphere at a low redox created by the oxygen demand of the organisms utilizing exudates from roots. Drying of the soil by plant growth or by air drying led to increased stability of soil aggregates presumably due to the increased sorption and effectiveness of organic binding agents, probably by polysaccharides which are exuded from roots in addition to the soluble exudates[30].

It is clear that as root systems mature and root hairs and roots are being decomposed continually, and the rhizosphere organisms establish themselves in root mucilage the stabilizing influences of root systems will be dominant. However, in soils which are cultivated regularly with low organic matter inputs, oxidation of organic materials will proceed at a rate which causes a gradual reduction of the organic matter content of the soil with resultant change in the composition of the residual organic matter[31]. There is an increase in the ratio of fulvic to humic acids. So the organic materials in the 'run down' soil are more soluble, more aromatic and possess more acidic functional groups per unit weight than those in a corresponding soil with 2 to 3 times the organic matter content. This loss of organic matter not only led to a substantial loss of water stable macroaggregates[52], but also a significant increase in dispersible clay probably as a result of oxidation of organic binding agents in addition to any effect of increased proportions of fulvic acids.

Flocculation and binding

The necessity to oxidise soil organic matter to determine the amount of clay in soils is well known. The standard practice during determination of inorganic particle size distributions by peroxidation of organic matter not only releases clay from microaggregates, but also releases large quantities of very fine clay, *e.g.* particle diameter ~ 10 nm. The specific surface area of clay fractions has been increased manyfold by oxidation of organic materials (Table 2). To account for these very large increases in surface areas of fine clay fractions either the organic materials coat aggregations of clay particles and render them inaccessible to N gas when it is used to determine surface areas, or fine clay is embedded in a matrix of organic matter.

Polysaccharides

One group of natural macromolecules which have received considerable attention in the last two decades because they are important as glues in soils are the polysaccharides. Carbohydrates represent about one quarter of soil organic matter much of it being derived from plant

Table 2. Release of fine clay by peroxidation of organic matter (from Burford *et al.*[3]; Turchenek and Oades[54])

Particle diameter (μm)	Surface area m²/g	
	untreated	H_2O_2 treated
Red-brown earth; Rhodoxeralf		
<2	16	77
0.4–2.0	24	54
0.1–0.4	39	76
<0.1	32	149
Black chernozemic; Haploboroll		
0.4–2.0	24	85
0.4–2.0 < 1.8 g/cm³	7	157
0.1–0.4	44	132
<0.1	6	211
Terra rossa; Palexeralf		
<2	50	176
Krasnozem; Paleustult		
<2	54	141

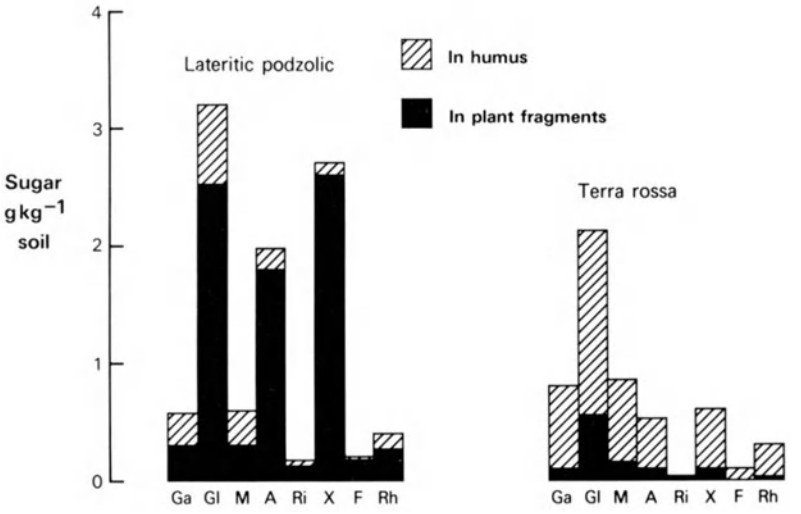

Fig. 4. Aldoses in plant debris and humus in an acidic and an alkaline soil. Ga galactose; Gl glucose; M maltose; A arabinose; R ribose; X xylose; F fucose; R rhamnose.

polysaccharides in roots and plan debris. The amount of the soil carbohydrate present in macroorganic matter (>¼mm sieve) or separated as a 'light fraction" by flotation or density procedures is substantial in acidic and sandy soils and much less in neutral to alkaline and clay soils (Fig. 4)[28]. These cellular plant polysaccharides are not involved directly in stabilization of soil structure. They are characterised by high proportions of pentose sugars.

The remainder of the polysaccharides in soils are mucilages associated with roots[30] or microbial mucilages[17]. Thus the rhizosphere becomes a major site for the production of mucilages, but they are also produced by organisms wherever organic debris is being decomposed. These mucilages are considered to be important glues in soils[4, 17, 27, 47] and evidence for this role includes correlations of aggregate stability and polysaccharide contents, the stabilization of aggregates by addition of microbial polysaccharides and polysaccharide preparations from soil, the degradation of aggregates by 'selective' oxidation of polysaccharides by periodate and recently the elegant identification of polysaccharides *in situ* in thin sections of soils[8, 9, 10, 11, 19, 20].

Recently Cheshire *et al.*[5] suggested that polysaccharides may be far more important glues than presently considered. They showed that 0.05 *M* periodate or prolonged oxidation with more dilute periodate caused further disaggregation of soils as more sugars were oxidised. However, the use of more concentrated periodate and long oxidation times raises the question of the selectivity of periodate for oxidising polysaccharides. When used on soils far more periodate is reduced than can be accounted for by oxidation of all the polysaccharides in the soil (Oades unpublished). Thus while there is no doubt that polysaccharides play a major role in binding soil aggregates determination of their precise contribution remains elusive.

Foster has indicated that the mucilages from fungi stain differently from those of bacteria. Otherwise the only guide to the origin of the mucilages in soil is the content of arabinose and xylose. Work with ^{14}C has shown that the soil microbial population synthesizes dominantly galactose, glucose and mannose and little if any arabinose and xylose, although at low temperatures yeasts produce xylose[4, 29, 35]. Plant materials contain substantial quantities or arabinose and xylose. Thus the ratio galactose + mannose/arabinose + xylose (g + m/a + x) is low (<0.5) for plant polysaccharides and high (>2.0) for microbial polysaccharides.

The composition of microbial polysaccharides is indicated in Fig. 5. Hexoses represent 70–90% of the aldoses, but equal amounts of amino compounds and uronic acids have usually been present (*e.g.* Swincer *et al.*[46]). Such complexes of macromolecules will react with clay surfaces by various mechanisms based on model systems which have been reviewed regularly (*e.g.* see Theng[49]).

The recent advances in the recognition of biological components by specific staining with heavy metals followed by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) have elucidated the relation between mucilages, associated with bacterial cells,

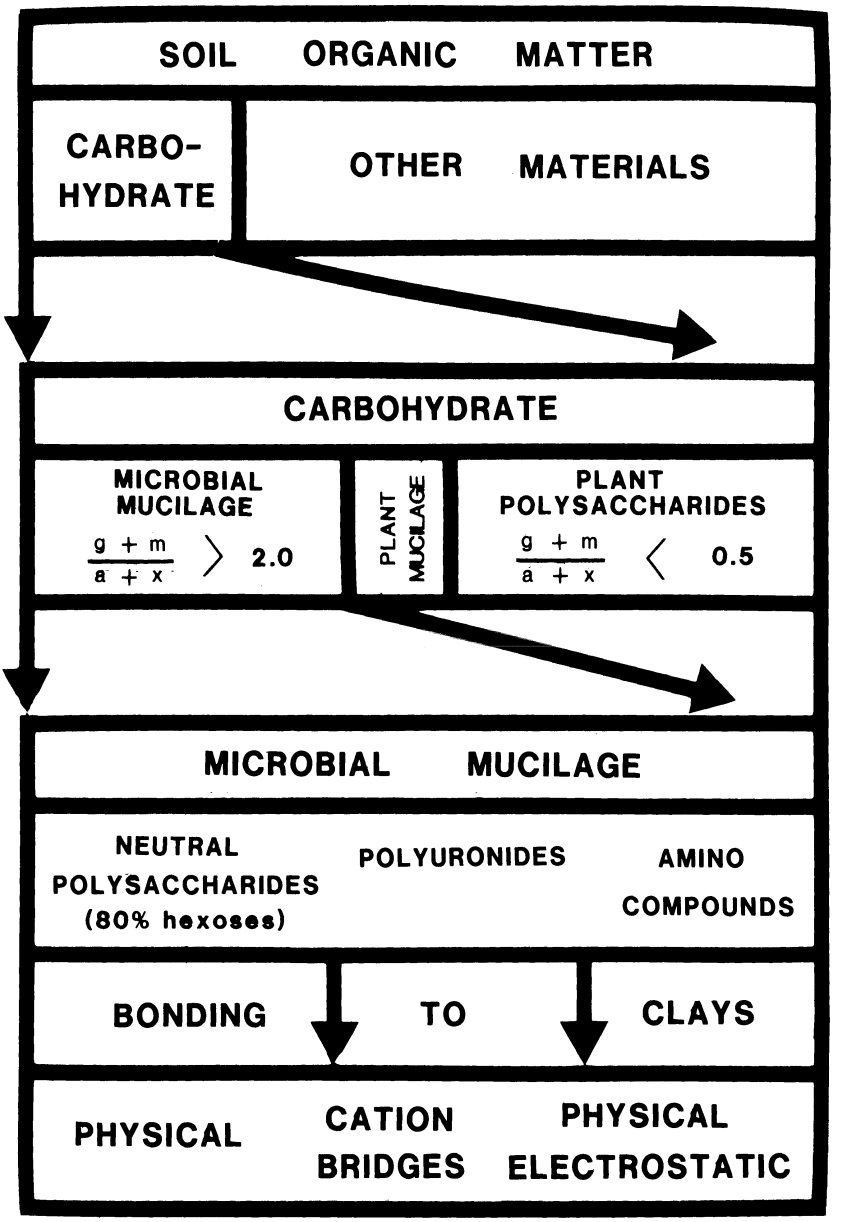

Fig. 5. Carbohydrates in soils.

fungal hyphae and the inorganic components, usually in the form of fine clay platelets[11, 19, 51]. Several important point arise from the electron microscopy

a) mucilages are produced *in situ* by roots or organisms and probably do not diffuse from the site of production. It is significant that the potential glues will be produced in pores accessible to roots and

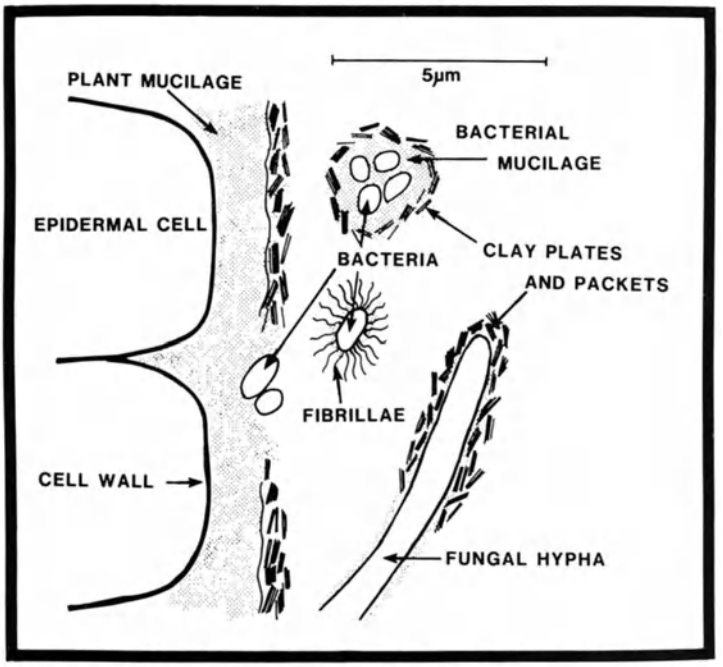

Fig. 6. Interaction of clay plates with mucilages.

organisms, *i.e.* the coarser pores from a few microns diameter upwards. These larger pores represent planes of weakness in aggregates whose stabilization is essential for structural stability.

b) polysaccharides exist as a gel or as fibrillae and do not move around in the soil as discrete polymers. The mobile component in the interaction between mucilage and clay appears to be fine clay. Dispersed fine clay will move with water in soil pores by gravity or matric suction towards roots until it contacts mucilages. The clay plates then seem to align themselves with the surface of the mucilages, encapsulating bacteria, surrounding fungal hypae or aligned parallel to roots (Fig. 6).

c) encapsulation of bacteria and associated mucilages by clay platelets and alignment of plates on mucilages indicates clearly why all the polysaccharide materials are not rapidly utilised by the microbial population. Much of the material is not physically accessible to organisms or enzymes. In addition, reaction of polysaccharides with metals inhibits degradation[27]. Thus, as Tisdall and Oades[53] described, while some of the stability of aggregates due to recently synthesised polysaccharides is transient because they are utilised by the microbial population, another part has considerable longevity. A substantial loss of organic matter

from soils can occur before the organic binding agents in microaggregates are degraded.

Cation bridges

Because clays and organic materials are polyanions they can be bridged by polyvalent cations. The cations normally considered are Ca, Mg, Al, Fe although others may be present in small amounts, *e.g.* Mn, Zn, Cu. While the divalent ions may exist as single ions with double charge which align water dipoles, Al and Fe are more likely to exist as hydrolysed species of indefinite composition and organisation, but with a net positive charge, *e.g.* polycations. The main evidence for the role of cation bridges has been based on increased disaggregation after treatments of soil with complexing agents or after treatments with acids (*e.g.* Hamblin and Greenland[15]). The complexing agents most commonly used have been pyrophosphate and acetylacetone, which is aimed particularly at Al and Fe. It has been shown consistently that pyrophosphate aids disruption of aggregates beyond that due to saturation of the system with Na. It is known to extract the more oxidised, aromatic components of the humic-fulvic system which have the most functional groups in close proximity and thus likely to complex with metal cations.[31, 55]. It also extracts some polysaccharides. The extraction of these organic polyanions is considered to be due to dispersion of the system and sequestering of polyvalent cations which have bridged clay and organic matter. The metal pyrophosphates are largely insoluble.

Pyrophosphate has been used to break cation bridges after periodate oxidation of polysaccharides[44] and caused further disaggregation. However, aggregate breakdown after sequential periodate and pyrophosphate treatments was always less than the sum of the aggregate disruptions caused by individual treatments of periodate and pyrophosphate on separate soil subsamples. The binding materials affected by both treatments could be the polysaccharides extracted by pyrophosphate and oxidized by periodate. Alternatively the common materials may involve the substantial polyuronide fraction. The pyrophosphate treatment gives no indications of the metals involved in the bridging effect.

Acetylacetone in aqueous and organic solvents has been examined as a means of breaking Al and Fe bridges between clays and organics[13, 14, 15, 16]. The limited results of the latter authors indicate that aqueous acetylacetone may be more efficient in extracting Al and Fe from organic materials. However, Giovannini and Sequi[13, 14] showed that acetylacetone in benzene led to a substantial decrease in the water stability of aggregates in five soils. They envisaged organic matter as a

network in which the junctions of the network were held together by Al and Fe. When the Al and Fe were removed wetting caused degradation of the net.

While pyrophosphate and acetylacetone may displace some organics from the surfaces of Al and Fe hydroxides, neither complexing agent is likely to displace polyanionic materials from oxide surfaces or from associations with highly disordered minerals. There are a number of good correlations between organic matter and disordered Al and Fe compounds[55]. Thus the influence of Al and Fe as bridges is probably underestimated.

Microorganism – clay interactions

Various sorptive interactions between soil particles and microorganisms have been reviewed by Marshall[26] who considered implications for the microorganisms rather than aggregation. More recently[2] the adhesion of microorganisms to surfaces has been described including the fundamental forces of interaction between organisms and surface and the implications for the organisms involved and the surfaces acting as adsorbent.

The soil microflora can be regarded as aquatic and it is reasonable to regard bacterial cells as part of the colloidal system that exists in soil. The fact that microorganisms are not readily leached or washed out from soils indicates that they are attached to either clay or humic materials and will be involved in dispersion–flocculation reactions which occur along with the other colloidal materials present. The cells are on average, larger than clay particles, they are ionogenic and in many cases amphoteric due largely to carboxyl and amino groups. Most microbial cells possess a net negative charge at the pH of the soil. It is therefore intriguing to consider how such negatively charged bioparticles approach clay surfaces sufficiently closely to be adsorbed. Organisms appear to offer at least two mechanisms to attach themselves firmly to clay surfaces: the first is the production of fibrillae which may extend up to tens of nm from the wall of the organisms to contact a nearby surface, the second is the production of a slime or mucilage which is dominantly polysaccharide in nature. While there is less information available for fungi many hyphal walls contain similar compounds to bacteria and similar properties could be assumed. Tisdall and Oades[51] presented some evidence that clay platelets were attached to fungal hyphae by means of a polysaccharide mucilage.

Several workers have shown that a substantial proportion of the soil biomass lives on or near the surfaces of aggregates, and while some can

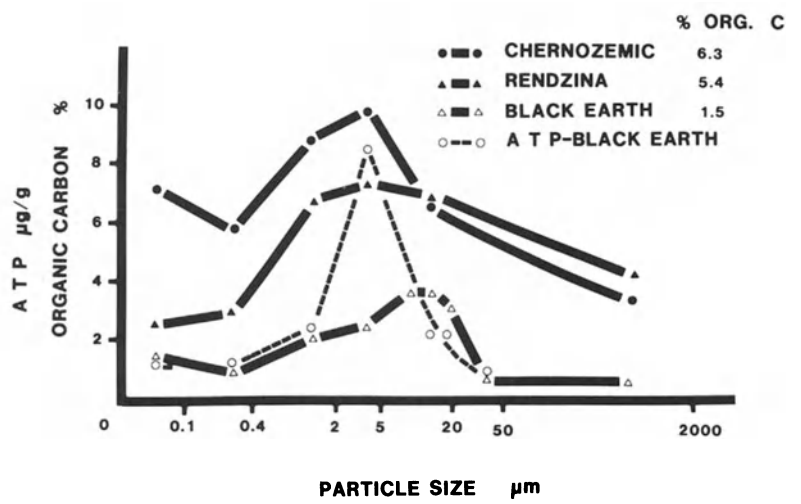

Fig. 7. Enrichment of carbon in aggregates in the silt fraction of calcareous soils.

be washed off by various procedures such as centrifugal washing, most remain with the inorganic fraction. When the aggregates are disrupted the microbial biomass is found in association with smaller particles particularly those from 1 to 20 µm equivalent spherical diameter, a proportion of which survive limited treatment with ultrasound. This appears to be particularly important in calcareous clay soils where organic materials and microorganisms accumulate in silt size particles resistant to dispersion by ultrasound[33, 48, 56]. Recently Ahmed[1] confirmed that substantial portions of the ATP in soil resided in the silt fraction. Furthermore, during the early stages of decomposition of glucose in soil substantial portions of the biomass (according to ATP) were present in a fine clay fraction. Subsequently there was a shift to larger particle sizes presumably as the organisms interacted with clay to form silt sized aggregates[1]. It is clear that the biomass which represents only several percent of the organic matter cannot account for the stability of all the aggregates in the silt fraction (Fig. 7) but that generations of bacteria have interacted with clay in a similar fashion forming a pool of stable aggregates enriched in carbon, nitrogen and also organic phosphorus[22, 23, 24, 34]. The fact that an accumulation of organic matter in silt fractions does not occur in leached acidic soils may reflect a much lower bacterial population, and/or perhaps a lack of calcium to bind organisms, residues of organisms and clay particles into silt sized aggregates.

Plate 1. Grass roots in black cracking clay.

Macroaggregates

Given sufficient quantities of mucilage or humic materials associated with metal ions spread uniformly through the soil, there is no reason why large aggregates cannot be stabilized. However, it seems that the production of a uniform matrix of organic materials is rare and slaking causes production of particles $100 \pm \mu m$ diameter. This is presumably a result of the bonds within the microaggregate being strong compared with those between the aggregates because of a discontinuous organic matrix caused initially by an uneven distribution of microorganisms.

However, it is clear that resistance to slaking is associated with larger pieces of organic debris from roots litter and fungi, or faecal pellets and worm casts, or from the network of living roots and hyphae.

The association of roots and hyphae with macroaggregates especially those > 2 mm diameter has been discussed by Tisdall and Oades[53]. The roots form a mesh with strands of diameter $10 \mu m$ upwards. These roots have grown in the coarser pores and are thus distributed around larger aggregates (Plate 1). Hyphae particularly the VA mycorrhiza can be regarded as an extension of this root system developing mycelia with hyphal diameters up to $10 \mu m$ throughout finer pore systems and around smaller aggregates (Plate 2). The fibrillae and flagellae of micro-organisms are another order of magnitude smaller and can occupy pores between clay packets and particles, *i.e.* stabilizing microaggregates.

The complex chemistry of roots and hyphae renders them resistant

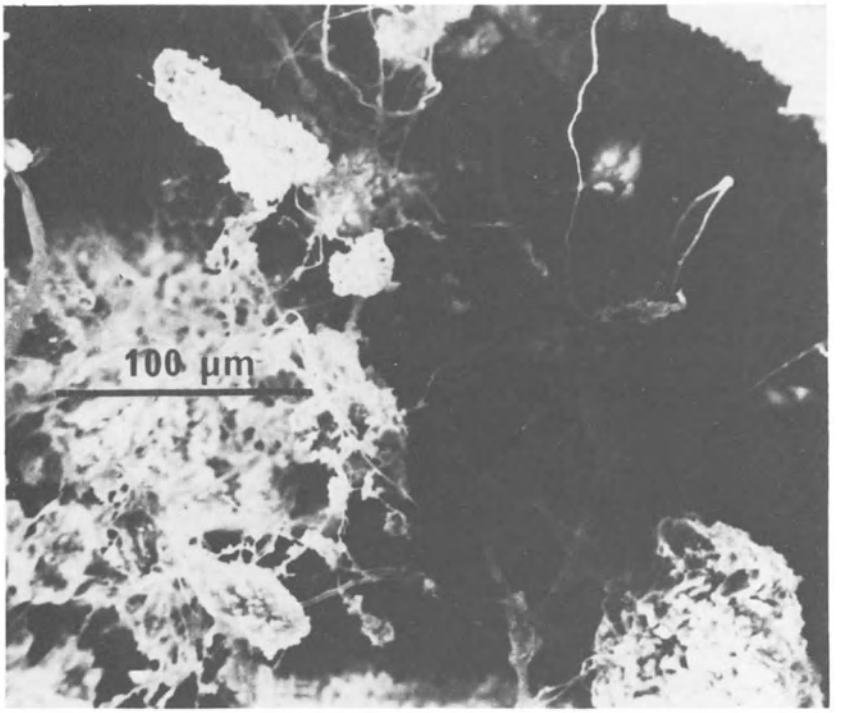

Plate 2. Scanning electron micrograph of soil particles entangled by fungal hyphae.

to chemical treatments, and the binding effect of the fungal hyphae, in particular, is not influenced by either periodate or pyrophosphate (Carter and Oades unpublished; Tisdall[50]).

The roots and associated hyphae have been described as temporary binding agents[53] because they do not persist for more than a year or so. Thus unless they are being replaced annually the binding effects disappear and the number of water stable macroaggregates declines. A bare fallow is notorious as the most deleterious management treatment with respect to soil structure.

As the roots and hyphae decompose the fragments become the centre of a water stable macroaggregate. Mucilages produced during decomposition of the organic fragment interact with clay which begins to encrust the organic fragment, eventually to an extent where the degradation of the organic material is retarded. The final product is a microaggregate bound by a matrix of humic materials and mucilages. This is a stage which exhibits considerable stability in the field unless there are chemical stresses which aid dispersion, *e.g.* exchangeable Na or severe mechanical stresses, *e.g.* raindrop impact.

Soil fauna

The influence of fauna on soil has been reviewed by Hole[18] and space does not permit an expansive treatment here, but a short statement is warranted.

Well aggregated soils always appear to contain a substantial population of both macro- and mesofauna. Whether the fauna are responsible for the porosity or whether suitable pores offer an optimal habitat for the fauna is a circular argument. Examination of calcareous soils under grassland indicates that surface layers are almost entirely materials 'casted' by earthworms or faecal pellets of other fauna including Collembola and mites. It is well known that regular cultivation decimates the numbers of macrofauna and it has been shown that certain herbicides have a similar effect on the Collembola and mites[25]. Very few approaches to management have considered the soil fauna, but stimulation rather than destruction of faunal populations may offer economical approahces to structural improvement, *e.g.* during stubble mulching or for improving infiltration rates from production of biopores.

Implications for management

Macroaggregation

In those soils where organic matter is the major binding agent macroaggregation is controlled by management. In general, numbers of macroaggregates can be increased by addition of decomposable organic materials. The best distribution of organic materials and mixing with the inorganic colloids is through root systems particularly the fine, bushy and extensive root systems of grasses. Limited data indicate that the stabilization of macroaggregates under grasses is maximal if the grass is cut at intervals which are long enough to allow root systems to recover. In this manner maximum death and growth of the root system is obtained, which will result in maximum organic inputs to the soil. Periods of bare fallow have the opposite effect. The proportion of a rotation which needs to be in grassland will depend on the soil and climate and will need to be determined for each situation.

The soil fauna will multiply given sufficient time, perhaps several seasons, with little soil disturbance. Addition of litter in appropriate circumstances will accelerate the recovery of the fauna, particularly earthworms. Infiltration of water has been increased manyfold by such treatments, but it is not applicable to broad scale farming.

Microaggregation

Microaggregation is not so sensitive to management. Therefore it is more difficult to improve microaggregation by normal farming practices although systems which conserve organic matter will slowly improve the number of microaggregates.

The pool of mucilages in the soil reflect an equilibrium between production by plants and organisms and degradation by subsequent microbial populations. Thus to maintain a large pool, stimulation of root growth is essential as this will give the best distribution of plant mucilage and energy source for microorganisms. Because grasses produce some 50% of their photosynthate below ground they are generally regarded as the most efficient 'structural improvers'. Alternatively, additions of organic matter will serve the same effect.

It seems unlikely that Al or Fe will be added to soils to stimulate bridging between organic materials and clays even though polycations of Al and Fe flocculate clays most efficiently[32]. However, the beneficial effects of maintaining a significant concentration of Ca in soil solution deserve examination. Gypsum can be used to supply sufficient Ca to flocculate colloids but unless large quantities are added the effect is only transient and it is not known if the high concentration of Ca for several years has any effect on the stability of polysaccharides, the production of Ca bridges between humic mateials and clays or on the stimulation of the soil microflora and fauna. Perhaps the long term effects of liming on soil structure should be reappraised.

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Influence of soil type, crop and air drying on residual carbohydrate content and aggregate stability after treatment with periodate and tetraborate

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Key words Aggregation Carbohydrate Crop Drying Periodate oxidation Soil

Summary The relationship between the water stability of microaggregates and the residual carbohydrate content of soil was examined in 15 soils from 7 soil series under various cultivations. The carbohydrate was progressively removed by increasing the time of treatment with 0.02 *M* periodate and 0.1 *M* tetraborate. The resulting decrease in reducing sugar content was significantly correlated with an increased disruption of microaggregates ($> 45\,\mu m$) as determined by a turbidimetric method. The most effective treatment removed about 80% of the soil carbohydrate and caused an increase of about 75% in the fraction of microaggregates ($< 45\,\mu m$) compared to untreated soil.

15–20 percent of the soil carbohydrate was resistant to oxidation by periodate, even after prolonged reaction times and contained a higher relative proportion of glucose, arabinose, and xylose than the oxidised material. Sugars typical of microbial sources, mannose, galactose, rhamnose and fucose, were therefore preferentially oxidised by the periodate treatment.

The grassland soils generally had higher carbohydrate contents than the arable soils and initially had a greater degree of aggregation. However, periodate oxidation affected each soil in its own characteristic manner. A significant inverse linear relationship between the degree of disruption and the residual sugar content was found with 13 of the 15 soils. Over the range measured aggregate stability was therefore related to the presence of carbohydrate predominantly from microbial sources.

Introduction

Treatment of a Countesswells series grassland soil with sodium periodate and tetraborate for increasing periods of time progressively decreased the residual carbohydrate content of the soil and caused disruption of microaggregates $> 45\,\mu m$; there was a highly significant linear relationship between these two effects[5]. The study has been extended to include other soils of various series under different cropping regimes.

Materials

Soils were chosen from seven different series in Britain (Table 1) to cover a range of clay and silt content as well as to compare the effects of grass and arable crop cover[1,8,9,11,12,22]

Table 1. Soil characteristics

Site No.	Site	Soil series	FAO soil group	Land use	Dried (D) Fresh (F)	pH	%C	%N	Sand > 20 μm	Silt 2–20 μm	Clay < 2 μm
1	Park grass plot 3A	Batcombe	Luvisol	Permanent grass	D	6.3	4.81	0.45	ND	ND	21
2	Broadbalk plot O3	Batcombe	Luvisol	Continuous wheat	D	8.0	0.93	0.10	51	25	24
3	Highfield	Batcombe	Luvisol	Ley-arable	D	6.5	4.00	0.38	45	35	17
4	Highfield	Batcombe	Luvisol	10 year fallow	D	5.4	2.47	0.22	46	31	23
5	Elgin golf course	Boyndie	Orthic Podzol	Permanent grass	D	5.2	2.94	0.26	95	2	3
6	Keam Duffus	Boyndie	Orthic Podzol	Arable	D	5.8	1.34	0.12	95	2	3
7	Macaulay Grounds	Countesswells	Podzol	Permanent grass	F & D	5.2	5.61	0.36	59	22	13
8	Meikle	Countesswells	Podzol	Arable, barley	F	5.8	3.02	0.23	66	27	7
9	Wanton Wells	Insch	Cambisol	Arable, barley	F & D	5.8	5.54	0.47	69	26	5
10	Westhall, Oyne	Insch	Cambisol	Grass	F	5.0	4.80	0.45	66	21	13
11	Burnton	Laurencekirk	Eutric cambisol	3rd year grass							
				0.5–2 mm	D	5.2	2.37	0.19	62	16	22
				< 0.5 mm	D	5.3	2.30	0.24	61	18	21
12	Burnton	Laurencekirk	Eutric cambisol	Arable							
				0.5–2 mm	D	5.3	2.61	0.26	83	7	10
13	Lower Powburn	Laurencekirk	Eutric cambisol	Potatoes	D	5.8	3.46	0.29	57	28	15
14	Shethin	Tarves	Cambisol	2nd year grass	F & D	5.4	2.90	0.33	58	25	17
15	Cruden Bay	Tipperty	Eutric cambisol	Permanent grass	D	5.8	3.56	0.29	51	26	23

ND – Not determined.

Fresh soil samples were < 6 mm sieved and either stored at 4°C in the dark or air-dried at < 80°C.

Methods

Sand, silt and clay contents were determined by the hydrometer method of Bouyoucos[2,3] after the soil had been dispersed by shaking for 16 h with 0.1 *M* NaOH.

Sugar analyses

Dried soil was ground by hand with a pestle and mortar and duplicate samples (0.1 g) were hydrolysed with 1 cm^3 cold 12*M* H_2SO_4 for 16 h followed by 0.5*M* H_2SO_4 at 100°C for 8 h. Total reducing sugar in the neutralized hydrolysates was determined with alkaline ferricyanide using a glucose standard in an automated procedure[4]. Individual sugars were determined by gas-liquid chromatography (GC) of the alditol acetate derivatives on a 1 m × 2 mm column of 3% SP-2340 on 100/120 mesh Supelcoport, using *myo*inositol as an internal standard[4]. Sugars were also measured using ion exchange column chromatography[4] to confirm the GC values.

Periodate treatment

One g samples of fresh or equivalent weights of unground dried soil were suspended without shaking in 30 cm^3 0.02*M* $NaIO_4$ or 0.02*M* NaCl for 6, 24, 48, 96 and 168 h at 22 ± 2°C in the dark. They were then centrifuged and the supernatant liquid decanted. The residue was resuspended in 30 cm^3 water and the mixture recentrifuged. After decanting, the residues were immediately mixed with 30 cm^3 0.1*M* $Na_2B_4O_7$ (pH 9.6). After 6 h the mixture was centrifuged, the supernatant liquid decanted and the residue washed with water by re-suspension and re-centrifuging. In the case of the 6 h treatment with 0.02*M* $NaIO_4$ or NaCl the samples were left for 16 h at 4°C before being mixed with 0.1*M* $Na_2B_4O_7$. For brevity only those results for 168 h are presented in Tables 2, 3 and 4.

The residue was then submitted to aggregate stability tests or evaporated to dryness at < 40°C. Sugars were determined on the dried hand-ground sample.

The whole procedure was repeated on a further set of samples of soils, 3, 7, 10 using 0.05*M* $NaIO_4$ or NaCl or water in place of the 0.02*M* $NaIO_4$, and water in place of the 0.1*M* $Na_2B_4O_7$. With the Macaulay soil 7, an additional treatment with 0.1*M* $Na_4P_2O_7$ for 24 h was applied after the 168 h 0.02*M* $NaIO_4$ and 6 h 0.1*M* $Na_2B_4O_7$ treatments .

Degree of aggregation

Soil samples (1 g fresh weight equivalent) were suspended in 50 cm^3 water in a stoppered glass tube (200 × 25 mm) and shaken in an end-over-end shaker for 25 min at 18 revolutions per min. The proportion of particles remaining in suspension and their rate of sedimentation were estimated turbidimetrically as described previously by Cheshire *et al.*[5]. To determine the 'total' disruption, the soil (1 g fresh weight or an equivalent dry weight) was sonically disrupted in 10 ml water using an MSE 100 W Ultrasonic Disintegrator for 5 min at 18 KHz. The suspension was made up to 50 cm^3 with water and shaken on an end-over-end shaker for 25 min and the resulting turbidity, expressed as $\log_{10}$ for reasons previously explained[5], was taken as representing 100% disruption. Control samples (taken as 0% disruption) received only the end-over-end shaking treatment. The regression equations (Table 4) were calculated using the data from periodate oxidation for 6, 24, 48, 96 and 168 h and sodium chloride treatment for 6 and 168 h.

Results

Effect of periodate on soil carbohydrate

The carbohydrate of all the soils studied showed a partial resistance to periodate oxidation as previously observed for a Countesswells series pasture soil[5]. Even after 168 h treatment with 0.02*M* $NaIO_4$, followed by 6 h in 0.1*M* $Na_2B_4O_7$, between 22 and 54% of the carbohydrate, measured as reducing sugar, and between 22 and 55 per cent of the

Table 2. Monosaccharide composition of the soils before treatment ($mg\,g^{-1}$) and the percentage in parenthesis of each sugar remaining after 168 h treatment with 0.02*M* $NaIO_4$ followed by 6 h 0.1*M* $Na_2B_4O_7$

Site No.	Glucose	Galactose	Mannose	Arabinose	Xylose	Fucose	Rhamnose	Total
1	5.7	2.9	1.9	2.5	2.6	0.53	0.87	17.0
	(73)	(29)	(42)	(80)	(38)	(28)	(33)	(55)
2	1.4	0.50	0.38	0.43	0.37	0.04	0.08	3.2
	(71)	(20)	(22)	(100)	(54)	(72)	(24)	(66)
3	4.4	2.3	1.8	1.7	2.0	0.38	0.74	13.3
	(41)	(14)	(17)	(62)	(22)	(19)	(12)	(31)
4	1.7	0.98	0.83	0.84	0.75	0.18	0.32	5.6
	(73)	(16)	(19)	(100)	(21)	(23)	(12)	(47)
5	5.0	2.0	1.3	1.6	2.5	0.32	0.55	13.3
	(29)	(8)	(12)	(37)	(20)	(10)	(12)	(22)
6	1.2	0.46	0.31	0.64	0.44	0.17	0.21	3.4
	(64)	(11)	(19)	(58)	(32)	(20)	(18)	(42)
7	6.5	1.9	1.8	1.9	2.1	0.35	0.84	15.4
	(65)	(28)	(30)	(56)	(45)	(25)	(26)	(49)
8	2.8	0.94	0.76	1.2	0.87	0.19	0.34	7.1
	(58)	(19)	(28)	(59)	(26)	(23)	(25)	(43)
9	3.8	1.9	1.5	1.5	1.4	0.38	0.69	11.2
	(50)	(18)	(20)	(52)	(31)	(19)	(16)	(35)
10	6.1	2.6	2.3	1.8	2.3	0.57	0.99	16.7
	(37)	(16)	(19)	(47)	(29)	(23)	(21)	(30)
11*	2.5	0.98	0.72	1.0	1.2	0.13	0.23	6.8
	(43)	(9)	(12)	(60)	(22)	(18)	(12)	(32)
11**	2.1	0.61	0.58	1.1	0.71	0.15	0.25	5.4
	(42)	(12)	(10)	(62)	(28)	(12)	(8)	(36)
12	2.2	1.1	0.88	0.99	1.0	0.15	0.28	6.6
	(43)	(13)	(14)	(76)	(21)	(31)	(32)	(35)
13	1.7	0.91	0.63	0.83	0.63	0.18	0.29	5.2
	(47)	(15)	(19)	(43)	(33)	(14)	(28)	(33)
14	3.7	1.7	1.3	1.5	1.6	0.28	0.57	10.6
	(28)	(12)	(17)	(36)	(11)	(28)	(15)	(22)
15	3.4	1.4	1.0	1.1	1.5	0.26	0.44	9.1
	(41)	(17)	(12)	(42)	(19)	(10)	(9)	(28)
Mean %	(49)	(17)	(21)	(65)	(30)	(25)	(20)	(40)
C.V. %	7.7	6.3	8.0	5.7	6.8	8.2	6.9	

* 0.5–2 mm; ** < 0.5 mm

Table 3. Effect of 0.02 *M* periodate treatment on the reducing sugar content (mg g^{-1}) and aggregate stability in soils. Figures in parentheses are the per cent disruption after the various treatments

Site No.	Untreated	Reducing sugar After treatment 168 h NaCl	168 h $NaIO_4$	Turbidity untreated	sonicated	Regression between reducing sugars (x) and disruption (y)	Correlation coefficient, significance[b] and number of samples
1.	19.8	17.4 (26.9)	9.9 (64.7)	3.91	1.14	y = 114.33 − 5.19x	−0.974 *** 7
2.	3.7	3.5 (52.9)	1.8 (73.2)	2.81	1.63	y = 87.43 − 7.58x	−0.790 * 7
3.	13.2	14.3 (46.7)	6.4 (73.3)	3.31	1.27	y = 97.20 − 3.83x	−0.974 *** 7
4.	6.5	6.5 (64.9)	3.4 (66.3)	2.81	1.37	y = 73.42 − 1.25x	−0.608 NS
5.	13.1	11.4 (10.6)	3.8 (55.9)	3.87	2.09	y = 75.80 − 5.88x	−0.965 *** 8
6.	4.0	5.0 (17.1)	2.0 (31.9)	3.84	2.46	y = 39.66 − 4.43x	−0.763 * 7
7.	16.5	13.7 (42.8)[a]	6.0 (74.0)	2.99	1.34	y = 87.93 − 3.43x	−0.920 ***20
	16.5	12.7 (35.0)	5.8 (48.3)	3.88	0.13	y = 70.51 − 4.09x	−0.983 ** 5
8.	8.5	6.3 (9.9)	2.9 (42.4)	2.88	1.71	y = 56.01 − 6.69x	−0.957 *** 8
9.	11.2	10.3 (47.2)	3.9 (60.0)	3.68	0.18	y = 67.59 − 2.08x	−0.968 *** 8
	11.2	10.5 (54.9)	3.0 (74.6)	3.81	1.05	y = 78.73 − 2.79x	−0.865 * 7
10.	15.4	12.3 (31.0)	5.7 (56.5)	3.08	0.48	y = 69.55 − 2.73x	−0.972 *** 8
11.	7.8	5.1 (75.9)	2.5 (81.3)	3.36	1.75	y = 85.55 − 1.81x	−0.886 ** 8
	7.3	5.3 (73.5)	2.5 (78.6)	2.92	1.70	y = 82.84 − 1.98x	−0.939 ** 7
12.	7.1	5.1 (77.6)	3.0 (88.3)	3.54	1.70	y = 109.05 − 7.06x	−0.948 * 7
13.	6.6	6.1 (73.7)	1.7 (87.3)	3.33	1.41	y = 95.11 − 3.38x	−0.966 *** 7
14.	11.0	10.0 (32.4)	4.2 (58.8)	2.61	0.77	y = 69.65 − 3.86x	−0.904 ** 8
	11.0	7.9 (60.4)	2.4 (74.7)	3.80	1.28	y = 78.46 − 2.52x	−0.913 * 7
15.	8.5	8.5 (59.3)	2.6 (66.7)	2.93	1.10	y = 69.46 − 0.80x	−0.646 NS 7

[a] Determined at 72 h

[b] *, **, *** Significant at $P < 0.05, < 0.01, < 0.001$ respectively; NS = not significant

individual sugars, determined by GC (Table 2), remained in the soil residues.

In most cases glucose, arabinose and xylose showed a greater persistence than galactose, mannose, fucose and rhamnose. The degrees of persistence of glucose and arabinose were similar for each soil and much greater than those for xylose: mean values, as a % of the original, were 49, 65 and 30 respectively. Of the other hexoses, mannose was usually more persistent than galactose. The overall mean value for all sugars was 40%.

Effect of periodate on soil structure

When soil was suspended in water for short periods, such as during the 25 min shaking procedure, there was a limited amount of disruption. This has been expressed in Table 3 as the turbidity of the untreated sample, a higher value indicating less turbidity and disruption. A comparison of fresh and dried samples of the same soils shows that drying greatly reduced the initial turbidity. Soils with a small clay content also showed little dispersion. For each soil the degree of disruption caused by the chemical treatments (y) was expressed by the equation

$$y = \frac{T_c - T_t}{T_c - T_s} \times 100$$

where T_c = the turbidity of the control sample; T_t = the turbidity of the treated sample; T_s = the turbidity of the sonicated sample.

Prolonged suspension in water caused further disruption. For example, with Cruden Bay soil, static suspension in water for 6 h increased the dispersion by 31%. However, the effect was not progressive and after 168 h the dispersion was unchanged.

Besides this effect of water there was a further effect of the ionic concentration of the reagents. Low concentrations, $0.01M - 0.1M$, of NaCl or $CaCl_2$ over a period of 30 min caused little change, but similar concentrations of $Na_2B_4O_7$, for example $0.1M$, were quite disruptive. Comparison of pretreatments of soil with water, $0.1M$ NaCl or $0.1M$ $NaIO_4$, prior to the $Na_2B_4O_7$, treatment, showed that with water and NaCl, the resulting dispersion could be attributed almost entirely to the $Na_2B_4O_7$ (Table 4). However, except with the $0.02M$ $NaIO_4$ pretreatment the effect was not progressive and there was little change in dispersion between 6 and 168 h.

The effect of periodate oxidation on soil carbohydrate appeared to continue throughout the duration of the treatment and was accompanied by a progressive disruption of the soil structure. A significant inverse linear relationship between the residual polysaccharide content

Table 4. Effect of pretreatment with water, 0.1*M* NaCl or 0.1*M* $NaIO_4$, for 6 or 168 h on the percentage disruption of three soils caused by treatment with 0.1*M* $Na_2B_4O_7$ for 6 h

	Countesswells (fresh)		Highfield arable		Westhall Oyne	
Pretreatment	6 h	168 h	6 h	168 h	6 h	168 h
Water	48	54	46	43	36	37
NaCl	42	43	41	47	35	31
$NaIO_4$	44	74	50	73	34	57

and the degree of disruption was obtained for 13 of the soils tested (Table 3). 2 soils, Highfield fallow and Cruden Bay grassland, showed no significant relationship: both were poorly aggregated and showed a high degree of dispersion even before periodate treatment. A directly proportional relationship between aggregate disruption and loss of polysaccharide would mean that 100% disruption should occur when the polysaccharide content was zero. In the majority of cases, however, the regression equations predict significantly less than 100% disruption when all the polysaccharide is removed, the overall mean value being 79%. The regression equation also predicts the initial polysaccharide content of the soil to be much greater than was found. This is because some dispersion is caused not by polysaccharide removal, but by the initial suspension and by ions in the reagents. Taking average values for all soil samples, some 47% of the disruption at 168 h is caused by 0.02*M* NaCl/0.1*M* $Na_2B_4O_7$ compared to 66% for the 0.02*M* $NaIO_4$/0.1*M* $Na_2B_4O_7$ treatment. Thus, on average, an extra 19% disruption was caused by removal of polysaccharide.

When Countesswells series soil which had been oxidized with periodate for 168 h was then treated with 0.1*M* pyrophosphate for 24 h there was only a small further increase from 74 to 77% in disruption and the carbohydrate content decreased slightly from 6 to 4.5 mg g^{-1}.

All soils showed some disruption of aggregates on treatment with periodate, but the extent of disruption did not appear to be related to the soil characteristics measured or cropping history. However, in two instances soils of the same series from different sites, (Wanton Wells and Westhall Oyne – Insch series; Highfield arable and Park Grass – Batcombe series), could be included in a single linear regression equation.

Discussion

The findings reported here are similar to those obtained in an earlier study by Cheshire *et al.*[5] on a Countesswells grassland soil. With all the soils the rate of oxidation of soil carbohydrate by periodate was very

slow and was still far from complete after 168 h. The shorter periods as generally used to remove polysaccharide in studies on aggregation[10,17,18,19,20] appear to be inadequate for the soils we have tested.

There are several possible reasons for the incomplete oxidation of the carbohydrate. For example, not all carbohydrate structures are susceptible to attack; 1–3 linked sugars lack vicinal hydroxyl groups and other substituents besides glucosidic linkages may prevent oxidation. Even those sugars present in 1–4 linkages which do possess vicinal cis hydroxyl groups may undergo partial oxidation to form hemiacetals[14,15] and these are stable to further attack. Furthermore, aggregates present in soils rich in Ca are relatively stable to periodate oxidation because Ca has an inhibitory effect on the reagent[6].

The oxidation of large fragments of plant materials is, in any circumstance, a slow process. In soil it is envisaged that the physical arrangement of substances within aggregates will be an additional hindrance.

When pyrophosphate treatment was used sequentially to periodate treatment, further aggregate disruption occurred[6,10]. This has been interpreted as evidence for the involvement of non-polysaccharide organic material in the bonding mechanism. However, those conclusions were based on the results of much shorter periodate treatments than in our procedure, which shows relatively little effect of pyrophosphate after extended periodate treatment of one soil. Some aggregates that depended on polysaccharide probably survived the shorter periodate treatments. Furthermore, pyrophosphate has also been found to be an effective extractant for soil polysaccharide[7,13] which may explain part of the effect of this reagent.

If polysaccharide is responsible for the remaining aggregation after an extended periodate treatment, the mechanism may differ from that of the periodate-sensitive material. The residual material certainly differs in its sugar composition and a contribution to the aggregation process by other soil substances must be highly likely.

Tisdall and Oades[21] state that polysaccharides are of less importance for aggregate stability in soils with many years of pasture growth. However, our work shows that in terms of resistance to periodate oxidation, soil aggregates from long-established pasture behaved similarly to those of arable soil. Preliminary results from more recent studies indicate that a sensitivity to periodate is also observed with larger aggregates up to 2 mm diameter.

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Changes in soil structure and biological activity on some meadow hay plots at Cockle Park, Northumberland

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Key words Biological activity Bulk density Grass Organic matter accumulation

Summary The accumulation of organic matter to form an organic horizon on one of the Palace Leas meadow hay plots has resulted from the effects of ammonium sulphate application over a period of 86 years. Associated with this accumulation is a reduction in biological activity, deterioration in hydrologic conditions and an increase in soil bulk density. The primary cause of the organic matter accumulation has been acidification. The worsening of hydrologic conditions as a result of the development of an organic horizon and soil structural deterioration may encourage further accumulation of organic matter.

Introduction

Palace Leas meadow hay plots were established in 1897 in order to demonstrate the effect of a range of fertilizers and organic manures on the yield of hay from long established grass. The manurial treatments have continued with only minor changes from 1897 to the present day. Effects of the manurial treatments on yield and sward composition were reported in the Annual Reports of Cockle Park Experimental Station (1897–1954) and by Coleman[3]. The development of a surface organic mat on some of the more acid plots was first described by Pawson[8]. Floate[4] quantified the redistribution of organic matter towards the surface layer of the more acid plots, but found no evidence for overall accumulation of organic matter on any of the plots. Arnold *et al.*[1] recorded differences in bulk density between the profiles on differently treated plots. During 1978 large pits were dug in Palace Leas field (for demonstration purposes) and accurate descriptions made of the soils on some of the plots (Payton, personal communication). These descriptions indicate that on the more acid plots there is evidence of greater mottling intensity and reduction in macropore space compared with the less acid plots. Although Palace Leas plots were established later than the Park Grass Experiment at Rothamsted, the site is in a wetter climatic region[10] where long term changes in soil properties are likely to be strongly expressed.

Table 1. Manurial treatment hay yield and pH of some of the Palace Leas meadow hay plots

Plot	Source of nutrients	Total nutrients supplied			Hay yield	pH (water)
		N	P	K		
		kg ha^{-1}			t ha^{-1}	
4	FYM applied every 2nd year	50	30	42*	4.23**	5.6
6	–	0	0	0	2.08	5.0
7	Ammonium sulphate	30	0	0	2.53	4.2
8	1897–1976 Basic slag 1977–1983 Triple superphosphate	0	26	0	2.86	5.8

* Average annual rate of application.
** 86 year mean.

Materials and methods

The manurial treatments, hay yields and pH of the plots selected for this study are shown in Table 1. All the plots are 120 m by 15 m and are unreplicated. Plots 6, 7, 8 are contiguous while plot 4 is separated by 15 m from the other three. The plots therefore lie within a rectangle 120 m X 75 m. The soil has been mapped by the Soil Survey of England and Wales (Jarvis, unpublished) and is of the Hallsworth Series.

Samples of soil bulk density and organic C content were taken from 0 to 27 cm depth, using Kubiena tins, from four sites 25 m apart in each plot, divided into 3 cm thick sections and the moist volume measured accurately. The mass of soil was found after oven drying (105°C). The samples were then broken up and the mass of stones > 2 mm recorded. The broken samples were ground by hand in a mortar to pass through a 149 μm sieve. The carbon content was then found using an induction furnace and Infracarb gas analyser.

For an incubation study two 50 mm diameter soil cores 55–60 mm long were taken at each of four sites 25 m apart on the plots. Each pair of cores was placed in a large glass jar with an airtight seal and incubated at 19–22°C. After an equilibration period of one week a beaker containing 20 cm^3 of 1 *M* NaOH was placed in each jar of soil in order to absorb the CO_2 produced from the soil. Beakers of NaOH were also placed in two empty jars to act as blanks. After one week the NaOH was titrated against 1 *M* HCl with a phenolphthalein indicator. The CO_2 produced was measured in this way for four weeks. The cores from the incubation experiment were then broken up by hand. To half of the jars 0.74 g $Ca(OH)_2$ (equivalent to 5t$CaCO_3$ ha^{-1}) was added, and mixed with the soil. The jars were resealed and left for a week. The beakers of NaOH were then replaced and the CO_2 produced was measured for a further two weeks.

For radiorespirometric study the method of Mayaudon[7] wqs used. Four samples were taken from each of plots 6, 7 and 8 at 5–10 cm depth and the moisture content was lowered to ~ 20% by air drying. 4 g of soil was then placed in a 50 cm^3 conical flask and 0.33 cm^3 of ^{14}C labelled glucose solution containing 30 μM glucose and having activity of 0.12 μCi cm^{-3} was added. The flask was sealed and placed in an incubator at 20°C. The $^{14}CO_2$ produced was measured using a scintillation counter.

The presence of significant differences between the results on the four plots was detected using ANOVA. Where such differences occurred the individual means were compared using a multiple T-test.

Table 2. Bulk density of soil on plots 4, 6, 7 and 8 at depths from 0 to 27 cm

Depth	Plot 4	6	7	8	SE
cm			$g\,cm^{-3}$		
0–3	0.65	0.56	0.28	0.74	0.032
3–6	0.88	0.94	0.54	0.94	0.037
6–9	1.02	1.15	1.13	1.13	0.028
9–12	1.03	1.22	1.29	1.16	0.028
12–15	1.18	1.29	1.30	1.27	0.030
15–18	1.33	1.31	1.46	1.39	0.038
18–21	1.37	1.36	1.36	1.39	0.037
21–24	1.44	1.41	1.42	1.45	0.053
24–27	1.47	1.51	1.47	1.50	0.050

Results

The bulk density of each 3 cm section of soil after allowance had been made for the stone content is shown in Table 2. Plot 7 had a significantly lower density than the other plots from 0–6 cm depth but from 9–18 cm depth its density was either similar to or significantly higher than that of the other plots. Plot 4 by contrast tended to have a lower density than the other plots from 6–15 cm. The density of plots 6 and 8 tended to fall between that of plots 4 and 7. From 18–27 cm depth no significant differences in density were detected between any of the plots.

The carbon content was measured on a gravimetric base, and because of the large differences in soil bulk density was converted to a volumetric base[4]. For each 3 cm thick section of soil the carbon content was found and finally a total carbon content for the whole 27 cm deep profile was calculated by summing over the individual sections (Table 3). Between 0 and 9 cm depth the carbon content of plot 7 was significantly higher than that of the other plots, whereas from 9–15 cm the carbon content of plot 7 was significantly lower than plots 4 and 6. From 15 cm downwards there was a tendency for the carbon content of plot 4 to be higher than that of plot 8. The top 27 cm of soil plot 7 was found to have significantly more carbon than the other three plots, also plot 4 contained significantly more carbon that plot 8.

The amount of CO_2 produced in the incubated soil cores during the four weeks of incubation is shown in Table 4, along with the mean of the four weekly readings. In all four weeks plot 7 produced significantly less CO_2 than plots 4 and 8 and less than plot 6 in three weeks out of four. Plot 6 produced less CO_2 than plot 4 in weeks two and

Table 3. The carbon content of soil on plots 4, 6, 7 and 8 at depth from 0–27 cm

Depth	Plot 4	6	7	8	SE
cm			$mgC\ cm^{-3}$		
0–3	51	42	101	50	6.1
3–6	53	46	105	45	3.9
6–9	49	40	59	40	1.9
9–12	39	39	33	35	1.5
12–15	37	34	27	30	1.7
15–18	34	29	31	27	1.9
18–21	26	27	25	26	1.0
21–24	25	22	22	21	1.9
24–27	22	20	17	16	2.2
			$mgC\ cm^{-2}$		
Total (0–27 cm)	1002	903	1262	863	25.7

three. The effect of liming was to significantly increase the CO_2 production from all the soils in both weeks of measurement. In the first week CO_2 production was increased by 18% due to liming and in the second by 36%. There was no significant difference between the amounts of CO_2 producted on the four plots, nor was there any significant interaction with the lime treatment. The rates of glycolysis measured in the radiorespirometry experiment were significantly different on all of the plots (Table 4).

Discussion

The redistribution of organic carbon towards the surface horizon of plot 7, as observed by Pawson[8], Barrett[2] and Floate[4], is confirmed. Not only is the organic matter content of the top 9 cm of plot 7 higher than that of the other plots, but also the content between 9 and 15 cm depth tends to be lower. As the top 6 cm of plot 7 has more than 18% C (gravimetric) it can be considered an organic horizon[5] and must have developed since the beginning of the demonstration in 1897. If this 6 cm thick organic horizon is discounted then 6–27 cm depth on plot 7 will be equivalent to 0–21 cm depth on the other plots. On this basis the organic carbon content of plot 7 below 9 cm (depth from present soil surface) will be lower than that of the other plots at an equivalent depth.

The total amount of organic matter in the top 27 cm of plot 7 is greater than that in the other plot indicating accumulation as well as redistribution. This accumulation, which amounts to an increase of some 40% over the control was surprisingly not detected by Floate.[4] If allowance is made for the depth of redistributed organic matter at

Table 4. Production of carbon dioxide (CO_2–C) and carbon accumulation on plots 4, 6, 7, 8

Plot	CO_2 produced in incubation					CO_2 production per unit of soil carbon	$^{14}CO_2$ production	Mean carbon accumulation rate*
	Week							
	1	2	3	4	Mean			
	μgCO_2–C cm^{-3} day^{-1}					μgCO_2–C gC^{-1} day^{-1}	μgCO_2–C day^{-1} cm^{-3}	t ha^{-1}
4	550	532	425	361	467	0.149	–	0.11
6	529	443	368	350	422	0.159	1090	0.00
7	475	400	307	293	369	0.059	360	0.41
8	539	493	414	354	450	0.158	980	– 0.05
(SE)	40	21	17	16			24	

*Relative to the control (plot 6).

the surface of plot 7 then the 27 cm of plot 7 should be compared with 21 cm of the other plots. This strengthens the case for a net accumulation of organic carbon and, in this case, plot 7 contains 162% of the control (plot 6).

The organic horizon at the surface of plot 7 is however active biologically. Barrett[2] considered the H layer to contain over 50% of coprolitic material and Standen[11] noted high populations of enchytraeids. The biological activity of the surface layer of plot 7, as measured by CO_2 evolution, is lower than the other plots. If the CO_2–C evolved from the four plots is compared with the soil carbon content then the relatively low activity of plot 7 in comparison to the other plots is enhanced (Table 4). The potential glycolytic power of the soils measured in the radiorespirometric experiment also indicates low activity on plot 7. Estimation of inputs of organic material to the plot is more difficult. MacFadyen[7] related input of soil organic matter to yields of grazed grass, but there are complications in the case of Palace Leas because of the variation in grass species between the plots and in the differential grazing resulting from unrestricted access by livestock to the aftermath. In the case of plot 4 the input of carbon in the form of manure can at least be measured directly. Comparing the relative inputs (as measured by hay yield) and output (measured as CO_2–C evolved) on plots 6 and 7 would support the view that carbon might be expected to accumulate on plot 7 rather than plot 6. By comparison however the input to plot 8 is higher than plot 6 while the output is not significantly different. The potential glycolysis on plot 6 is, rather surprisingly, higher than on plot 8. The input to plot 4 consisting of both farmyard manure and the substantial grass growth suggests that the output should be considerably higher than plot 6 if carbon is not to accumulate rapidly. This is not supported fully by the incubation data, although Bluck (unpublished) found on Palace Leas plot 2 (treated annually with $20\,t\,ha^{-1}$ of farmyard manure) that the $^{14}CO_2$–C respiration was $1680\,\mu gCO_2$–C $day^{-1}\,cm^{-3}$, a figure significantly higher than that of plots 6, 7 or 8. The net accumulation rates of carbon are shown in Table 4 (assuming uniform carbon accumulation over the 84 year period). They are calculated relative to the control (plot 6) and suggest that the carbon content of plots 4 and 8 is close to being in equilibrium while only plot 7 shows a strong tendency for carbon accumulation.

The low CO_2 output from plot 7 relative to the other plots could be due to acidity or to wetness. Shaw[9], who investigated the surface organic mats on soils at Park Grass Experiment (Rothamsted) and from Rivington Series, found that CO_2 production was significantly increased

by liming. At Palace Leas liming also increased CO_2 production on all the plots. The effect of disturbing the soils had an apparent effect on CO_2 production, and the lack of significant differences between the four plots after both disturbance and liming suggests that the CO_2 production from plot 7 is very sensitive to changes in environment.

The differences in soil structure in plot 7 noted by Payton (personal communication) and Barrett[2] suggest a change to wetter conditions on this plot. Both authors noted a relative increase in mottle density in the horizons immediately below the organic mat. Changes in the structure noted in thin section (Daintree, unpublished) also suggests a reduction in macroporosity (pores $> 200\,\mu m$ diameter) on plot 7 and a trend towards platy structure on this plot. The bulk density measurements (Table 2) also indicate a rise in density in plot 7, relative to the other plots, immediately below the organic mat. If, as in the case of the organic carbon content, the bulk density is considered in relation to the original surface of the mineral soil then plot 7 shows a consistent increase in density over the other plots, below the organic mat. Assuming that all the plots had a similar density at the outset of the Palace Leas demonstration then the density of the original A horizon of plot 7 has increased sharply and this is associated with a fall in organic matter content of the mineral soil. Relating bulk density to carbon content a highly significant ($r = -0.99$) negative linear correlation (1) was found between density (BD) and carbon (OC) on plots 4, 6 and 8.

$$BD(g\,cm^{-3}) = 1.62 - 0.134\%OC\ (gravimetric) \qquad (1)$$

This is similar to the relation found by Williams[12] for a range of grassland soils. On plot 7 a negative curvilinear relationship (2) was found to fit the data better ($r = -0.99$) over the range of carbon from 1.2 to 36.0% (gravimetric).

$$BD(g\,cm^{-3}) = 1.62 - 0.82 \log \%OC\ (gravimetric)$$

The two regression lines are only significantly different over the range of values associated with the top 9 cm of plot 7. In this upper horizon the bulk density is relatively high in relation to its carbon content. The increase in mottling, reduction in macroporosity, changes in structure and increase in bulk density on plot 7 all indicate a worsening of hydrologic conditions. The cause of this worsening can only be attributed to the effect of the treatment applied to the plot, as the contiguous plots show no such effects. The reduction in pH, which has resulted in soil faunal changes already noted, floral and nutritional changes in the herbage, and the reversal of which increases CO_2 production, appears to have led to structural changes in the soil which have

produced an increase in wetness. Together the acidity and wetness have led to an accumulation of organic matter.

Reversal of the accumulation could be easily achieved by liming if the only problem were acidity. The poor hydrologic conditions on plot 7 suggest that in addition to liming, improvements in soil structure would be necessary. To achieve such changes rapidly cultivations would be required. The finding that soil disturbance improves CO_2 production on plot 7 also indicates the potential value of cultivation in improving this soil. The depth of cultivation to reduce density on plot 7 would need to be approximately 20 cm from the present surface as densities below this depth are high on all the plots.

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The effects on topsoil of long-term storage in stockpiles

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Key words Aggregate stability Anaerobism Biomass Earthworms Restoration Soil atmosphere Stockpiles Storage Topsoil VAM Vesicular-arbuscular mycorrhiza

Summary During many quarrying, civil engineering and landscaping schemes topsoil is stripped from the site and stored in large heaps. These remain *in situ* for many years before the soil is reused and it is generally believed that there is a great reduction in the 'quality' of the soil during that period.

A study of stockpiles of different size, age and soil type has revealed that biological, chemical and physical changes do occur, mainly as a result of anaerobic conditions within the heaps, but also as a result of mechanized handling during the stripping and stockpiling. Visible changes occur within 0.3 m of the surface of stockpiles of clayey textured soils, but only below about 2 m depth for sandy textures. These visible changes are accompanied by chemical changes, particularly in the forms of nitrogen present but also in the content of available nutrients, pH and organic matter levels. Biological changes include reductions in potential for mycorrhizal infection, soil biomass and especially earthworm population. The soil atmosphere contains high levels of carbon dioxide, methane, ethane and ethylene. Physical changes include reduction in aggregate stability and resistance to compaction, increase in bulk density and changes in pore size distribution and micro-structure, as revealed by scanning electron microscopy.

Limited evidence suggests that many of the adverse effects quickly disappear when the soil is respread.

Introduction

Soil, especially topsoil is often saved from sites about to be disturbed, and placed in stockpiles for subsequent reuse in landscaping or restoration work. It is generally believed that in addition to the damage caused by mechanised handling and the compaction by heavy earth-moving machinery, there is a deterioration in the quality of the soil during storage. Hunter and Currie[5] considered that anaerobic conditions in topsoil heaps had affected organic compounds concerned in soil aggregation. O'Flanagan *et al.*[12], however, found relatively little structural deterioration and although rates of nitrification and counts of aerobic bacteria decreased with storage, there was a rapid recovery when the soil was respread. American workers, *e.g.* Miller and Cameron[10]; Singleton and Williams[16]; Reeves *et al.*[14]; Rives *et al.*[15] have concentrated attention on biological changes and have found, in particular, that

long-term storage of topsoil causes substantial reductions in the population of viable vesicular-arbuscular mycorrhiza (VAM).

Eighteen topsoil storage heaps were examined representing a range of textures, ages and conditions of construction and storage. Samples removed from various depths within the heaps were compared with similar topsoils on adjacent, unworked land. A range of physical, chemical and biological determinations was carried out. The most significant of these are presented here for three characteristic heaps representative of soils of sandy texture (Charing Heath, Ashford, Kent), loamy texture (Bush Farm, Upminster, Essex) and clayey texture (Haywarden Farm, Tonbridge, Kent). Ages of these heaps was from $1\frac{1}{2}$ years (Tonbridge) to over 7 years (Charing Heath), and heights of stacking varied from 3 to 7 m.

Methods

Soil atmospheres were collected by the method of Dowdell *et al.*[4] and analysed by gas chromatography. Aggregate stability was measured according to MAFF[9]. Standard chemical tests were carried out according to procedures described by MAFF[8]. pH was determined in a 1:2.5 soil:water suspension and organic matter by the Walkley–Black method. Phosphate was extracted by 1*M* $NaHCO_3$ solution at pH 8.5, potassium and magnesium by 1*M* NH_4NO_3 and manganese by 1*M* NH_4 acetate. The method of Jackson (1958) was used to extract iron with 1*M* NH_4 acetate. NH_4^+ and NO_3^-–N were extracted with 2*M* KCl. Earthworm populations were obtained by drenching soil with formaldehyde according to the procedure of Raw[13] and microbial biomass was obtained by the method of Jenkinson and Powlson[7]. The mycorrhizal population was investigated on the sandy soils of the Charing Heath site by the bioassay method of Moorman and Reeves[11] with samples from the surface of the undisturbed soil and from a depth between 1.8 and 2.0 m in the stockpile. A pot test growing lettuce was set up using undiluted soil and soil diluted with potting compost at $\frac{1}{4}$ and $\frac{1}{40}$ dilution. Each treatment was replicated five times. Plants were harvested 30 days after sowing and the percentage infection of the roots with vasco-arbuscular mycorrhiza (VAM) was calculated according to Daft and Nicholson[3].

Results and disucssion

Physical changes

The most obvious change in stockpiled topsoils was that the soils

Table 1. Soil atmospheres in stockpile heaps

Site	Depth m	Methane ppm	Ethane ppm	Ethylene ppm	O_2 %	N_2 %	CO_2 %
Charing Heath	0.5	.240	0.73	0.01	20.6	78.6	0.43
(CH)	2.0	2.45	1.01	0.17	19.1	78.9	1.96
Bush Farm	0.5	4.41	5.99	0.08	19.9	76.9	0.89
(BF)	1.5	4.65	3.72	0.18	18.6	78.7	2.31
Tonbridge	0.3	4.92	1.37	0.15	21.2	77.1	0.51
(T)	1.0	4.72	1.86	0.19	19.8	78.6	3.81

became dark grey in colour and gave off a characteristic anaerobic smell at depths from the surface which varied with soil texture. In the sandy Charing Heath soil the visual change occurred at about 2 m from the surface, whereas it was at about 1.3 m in the loamy Bush Farm soil and at only 0.3 m depth in the clayey Tonbridge heap.

The anaerobic nature of the heaps was also shown by analysis of soil atmospheres (Table 1). CO_2 and ethylene contents increased with depth and high levels of methane and ethane were found even relatively near the surface.

Compared with undisturbed soils, aggregate stability, measured on 1–2 mm aggregates, was greatly reduced by storage in the sandy and clayey heaps (Charing Heath and Tonbridge) but there was little effect on the loamy soil (Bush Farm) as shown in Table 2. Supplementary tests on the resistance of the soils to compaction under applied pressure at various moisture contents showed that stockpiled soils compacted slightly more at equivalent pressures and moisture contents than unworked soils. The corresponding reduction in the number of coarser pores, important for soil drainage, suggests that stockpiled soils are likely to be more susceptible to structural damage during subsequent respreading than they were before being stripped.

The microstructure of unworked and stockpiled soils was examined by scanning electron microscopy[1]. Loss of microstructure in stockpiled soils is particularly marked, even in the loamy Bush Farm soil. It is, however, uncertain how much of this damage is due to stockpiling *per se* or to the effects of compaction by heavy earth-moving machinery at the time the heaps were constructed. Bulk densities of surface soils showed an increase from unworked to stockpiled soils near the top of the heaps of the order of 1.5 to 1.8 for the loamy soil and 1.1 to 1.3 for the clayey soil. Bulk densities could not be determined accurately at depth in the heaps because of sampling difficulties.

Table 2. Aggregate stability (1–2 mm) of unworked and stockpiled soils

Charing Heath		Bush Farm		Tonbridge	
Soil depth m	Aggregate stability %	Soil depth m	Aggregate stability %	Soil depth m	Aggregate stability %
Unworked	34.4	Unworked	8.6	Unworked	95.7
0–0.2	2.6	0–0.2	8.1	0–0.2	77.9
0.4–0.5	1.1	0.2–0.3	5.4	0.2–0.3	82.3
0.7–0.8	0.6	0.3–0.4	8.1	0.3–0.4	61.9
0.8–0.9	1.7	0.4–0.5	9.2	0.4–0.5	49.4
0.9–1.0	2.0	0.5–0.6	6.2	0.5–0.6	60.8
1.0–1.25	0.7	0.6–0.7	6.3	0.6–0.7	50.6
1.25–1.5	1.0	0.7–0.8	13.0	0.7–0.8	45.7
1.5–1.7	6.3	0.8–0.9	18.4	0.8–0.9	43.0
1.7–1.8	6.3	0.9–1.0	2.8	0.9–1.0	39.5
1.8–2.0	15.9	1.0–2.0	3.7	1.0–1.5	33.9

Chemical changes

The chemical properties of samples from the three stockpiles and corresponding unworked land are given in Table 3.

The anaerobic nature of the heaps is most clearly shown by the contents of extractable manganese and ferrous iron. In the clayey Tonbridge heap, for example, ferrous iron rises from 72 to 5800 μ6/g at the point where the visible colour change in the soil occurs, and continues to rise with depth. In the sandy soil (Charing Heath) the sudden rise in iron content occurs at a much greater depth – around 1.5 m, just above where the colour change occurs. Results for the Bush Farm heap are much more variable and show no consistent trend with depth. Indeed, the results suggest the heap may be more heterogenous than was supposed. Corresponding trends are shown by manganese but in this case the results for the Bush Farm heap also show increased levels and hence probably increased anaerobism with depth. Tests on an adjacent heap at Bush Farm[2] showed that a high level of ferrous iron (1028 μg/g) in a composite sample taken from the core of the heap during respreading had dropped to only 124 μg/g in the respread soil two weeks later.

There is little variation in the amount of NO_3–N with depth in the stockpiles and levels are similar to those in unworked soils, analysed at the same sime. NH_4–N, absent or at a low level is unworked land, rises with depth in all stockpiles almost certainly because of the increasing anaerobism. The increase in pH in the stockpiles corresponds with the build-up of ammonium.

Extractable P, K and Mg tend to increase in the clayey Tonbridge stockpile, but to decrease slightly in the sandy (Charing Heath) and loamy (Bush Farm) stockpiles. In all cases, however, the levels are quite

Table 3. Some chemical properties of unworked (U) and stockpiled (S) soils – Charing Heath (CH), Bush Farm (BF) and Tonbridge (T)

Soil depth (m)	pH (1:2.5)	Organic matter %	Extractable elements μg/g						
			P	K	Mg	NH_4^+ N	NO_3^- N	Mn	Fe
CH(U)	5.3	1.9	34	162	100	0	3	5	228
(S)–0.2	6.5	0.7	19	61	24	3	1	7	534
0.4–0.5	7.0	0.3	21	30	23	2	2	7	371
0.7–0.8	6.6	0.5	19	30	23	2	2	7	323
0.8–0.9	6.5	0.6	24	30	22	3	2	20	133
0.9–1.0	6.2	0.7	30	30	28	4	1	32	157
1.0–1.25	6.5	0.4	24	30	26	4	2	55	155
1.25–1.5	6.4	0.7	29	30	28	13	1	139	697
1.5–1.7	6.5	0.8	32	30	36	19	2	164	4800
1.7–1.8	6.5	0.8	36	40	34	24	2	138	5280
1.8–2.0	6.4	1.1	34	70	41	50	2	169	6310
BF(U)	6.8	3.9	158	255	110	0	6	0	181
(S)0.02	6.8	3.1	140	193	88	1	3	0	43
0.2–0.3	6.9	2.9	156	193	89	10	8	5	132
0.3–0.4	7.1	2.7	153	183	95	17	10	5	23
0.4–0.5	7.0	2.9	161	214	107	26	9	12	90
0.5–0.6	7.1	2.9	145	183	102	30	30	15	136
0.6–0.7	7.0	2.8	132	214	105	25	26	7	91
0.7–0.8	7.0	3.7	171	246	97	105	8	12	233
0.8–0.9	6.9	3.8	163	285	88	117	6	15	301
0.9–1.0	7.1	3.2	130	173	79	77	9	7	164
1.0–2.0	7.1	2.8	130	194	97	50	9	7	197
T(U)	6.4	8.9	13	75	181	2	12	16	0
(S)0.02	6.2	7.9	36	169	271	3	11	16	117
0.2–0.3	6.5	8.1	48	158	265	9	21	42	76
0.3–0.4	6.5	8.3	51	189	281	177	17	52	72
0.4–0.5	6.3	7.2	40	168	254	364	18	52	5800
0.5–0.6	6.3	8.1	36	147	228	407	22	42	7035
0.6–0.7	6.4	7.1	35	137	229	422	25	42	13893
0.7–0.8	6.4	7.4	33	126	237	404	24	42	15860
0.8–0.9	6.5	6.1	31	116	245	307	20	74	17089
0.9–1.0	6.5	6.6	29	116	246	246	19	87	14000
1.0–1.5	6.5	6.6	28	126	260	259	22	92	18121

acceptable for agricultural purposes and so it could not be said that stockpiling had adversely affected chemical fertility, except in the case of nitrogen. On land restored with stockpiled soils nutrient defficiencies are rarely encountered but higher levels of nitrogenous fertilisers are usually recommended. Liming respread soils is required only when pH values are low.

Probably the most significant change due to stockpiling is a reduction in organic matter content: in the sandy heap (Charing Heath) up to 85% of the organic matter originally present has been lost and up to 32% in the other two soils.

Biological changes

Stockpiling has an adverse effect on the earthworm populations. The Results in Table 4 for earthworm number and biomass, both fresh and dry weights, are significantly lower ($p < 0.05$) for stockpiled soils than for adjacent unworked land.

Soil biomass, excluding earthworms, is given in Table 4 and similarly shows significant reductions in the stockpiles.

The mycorrhizal populations of the sandy soils of the Charing Heath site are given in Table 5. Storage has substantially reduced VAM infection, as found by the American workers quoted above. The $\frac{1}{4}$ dilution and $\frac{1}{40}$ dilution both showed that infection in unworked soil was approximately six to ten times greater than in the stored topsoil. Nevertheless the stored soil still retained sufficient mycorrhizal population to produce a 67% infection of the lettuce roots.

Table 4. Earthworm populations and biomass contents

Site	Soil	No. of earthworms	Earthworm biomass g/m²		Microbial biomass mgC/100 g dry soil
			fresh weight	dry weight	
Charing Heath	Unworked	79	225.0	45.6	8
	Stockpile	17	52.3	8.7	6
Bush Farm	Unworked	64	276.4	44.3	365
	Stockpile	40	90.5	13.1	307
Tonbridge	Unworked	141	159.0	28.9	1435
	Stockpile	20	26.5	4.0	1254

Table 5. VAM infection of bioassay plants grown in unworked and stored topsoil (Charing Heath site)

Treatment	Per cent mycorrhiza infection, mean*	
Unworked soil		
undiluted	79.3	a
$\frac{1}{4}$ dilution	50.0	c
$\frac{1}{40}$ dilution	19.1	e
Stored topsoil		
undilited	66.6	b
$\frac{1}{4}$ dilution	5.4	d
$\frac{1}{40}$ dilution	3.4	d

* Values followed by the same letter are not significantly different ($p < 0.05$)

Conclusion

It is well known that mechanised handling can seriously damage soils and it is difficult if not impossible to separate the effects of compaction by earthmoving machinery from those due to storage *per se*.

Unfortunately no heaps were available which were created by techniques such as the dump truck and back-acter method whereby soil is not compacted by machinery. Also it was very evident in some of the heaps that subsoil of totally different character had been incorporated, and there were pockets of extremely anaerobic soil associated with the burial of vegetation along with the soil.

Nevertheless, it is clear that while adverse effects due to storage can be demonstrated, the extent of deterioration of soil in stockpiles has been greatly overestimated. There is no reason why soils should not continue to be stockpiled and subsequently reused, though perhaps greater care should be taken to minimise compaction by earthmoving machinery, to avoid mixing topsoil and subsoil and to prevent loss of, or contamination of, soil while in store. Recommendations regarding the optimum size and shape of stockpiles are being prepared.

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Section 7

Effects of noxious materials on biological processes in soils

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Effects of pesticides and heavy metals on biological processes in soil

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Key words Assessment Distribution Heavy metals Impact Input Microbial processes Pesticides Side-effects Soil

Summary The side-effects of pesticides and metal cations are reviewed with regard to input, distribution, persistence and toxicity. The sensitivity of microbial processes is discussed in relation to adaptability and resistance of microbial populations. Procedures for an ecological assessment of toxic side-effects are discussed.

Introduction

In recent years we have become increasingly aware that the environment is endangered not only by single activities of relatively high risk but also by the cumulative effect of operations which we assumed to be of low residial risk. Over the last three decades, the monitoring of pesticide behaviour in soil and, in particular, that of side-effects on non-target organisms have become established disciplines in soil research. Nevertheless, uncertainty about the potential residual risk has continued to exist. Presumably it is for this reason that the state-of-art is reviewed and revised with increasing frequency. Although this review is the latest of many it is still difficult to recognize true advances related to current problems. A reiteration of familiar approaches and general conclusions is certainly not warranted unless we reexamine the wealth of information to establish where the problems remain and what research is needed.

During the last 45 years at least 1,580 papers have been published concerning side-effects of pesticides on soil microorganisms, until recently at a rate of 70–80 per year (Fig. 1). But quantity alone does not guarantee a minimization of environmental risks and, except for new ecotoxicological data we are obliged to ask for new approaches, new concepts, new techniques. The most important needs are more intelligent interpretations and the recognition of general principles as these become intelligible. Five years have passed since Hill and Wright[7] summarized the knowledge then available. In this report Anderson[1]

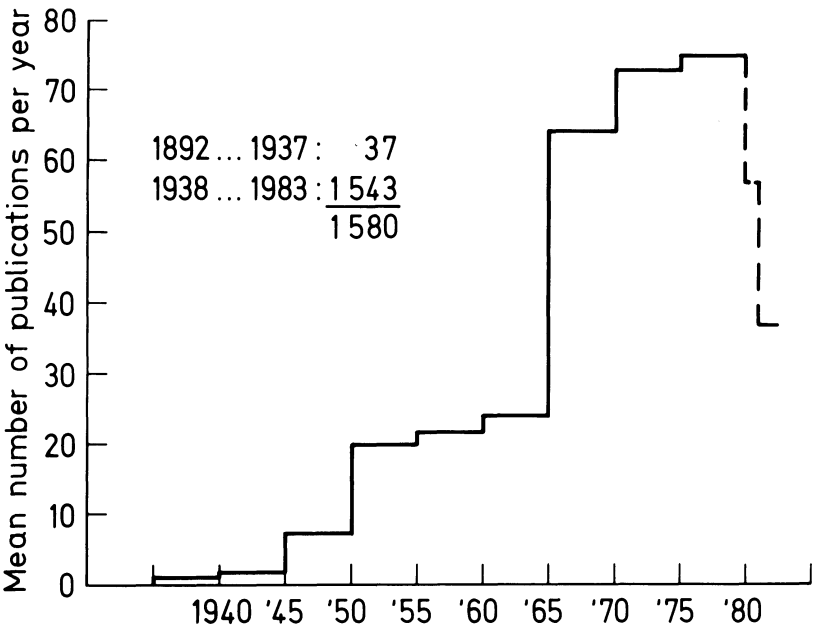

Fig. 1. Publications on side-effects of pesticides on soil microorganisms over the last 45 years

contributed a relevant 120 page table on the side-effects of pesticides on the microflora and microbiological processes in soils and concluded by listing some of the more pressing questions at that time. They are repeated here in a slightly modified version:

(1) Are the 11 or 12 aspects which are most frequently investigated selected with sufficient care, considering the numerous factors which are involved? Has some important factor been overlooked?

(2) Do conflicting results arise from inconsistent methods, soil heterogeneity, or varying climatic conditions? What can be learned from deviating results?

(3) Are we prepared to evaluate toxicological data on the grounds of firm ecological knowledge? How transient should an effect be to qualify as unimportant?

(4) Are interactions adequately investigated? What is the place of bacterio- and actinophages, mycoparasitism, nematode trapping fungi?

(5) Are soil microbiologists delving deep enough?

Evidently these questions are not identical with those posed at the onset of pesticide microbiology. In the following sections a modest attempt will be made to look for new answers to some of the more 'classical' questions. The analysis will consider pesticides and,

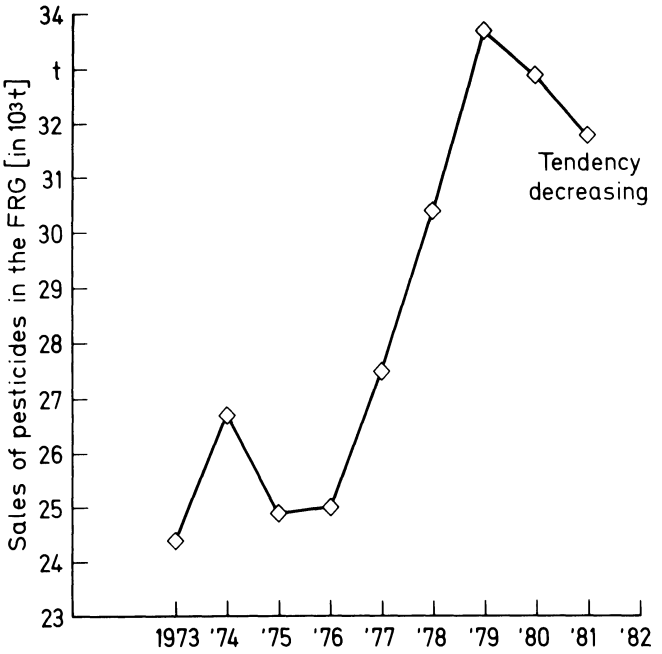

Fig. 2. Sales of pesticides in the Federal Republic of Germany since 1973 (from Statistische Jahrbücher über Ernährung, Landwirtschaft und Forsten 1976–82)

additionally, will examine heavy metals as a further group of agents potentially toxic to the soil microflora.

Pesticides

Input

This group of chemicals is commonly introduced by figures on annual world usage (*ca.* $10^6\,t\,a^{-1}$) and remarks on the number of parent substances and commercial products. Since 1980, however, we recognize a tendency for a decrease in the quantities of pesticides in the Federal Republic of Germany (Fig. 2). It is not yet clear whether economic or ecological considerations are responsible for this trend.

It should be stressed that the actual quantity of pesticide applied per unit area is small, usually far below $10\,kg\,ha^{-1}$, as long as the user follows the recommendations and restrictions given for registered pesticides. In contrast to the rather persistent chemicals of earlier generations, the newly released pesticides have considerably shorter half-lives in soil. Examples given in Fig. 3 indicate that this time is well below 180 days even under unfavourable soil and climatic conditions; thus preventing the build-up of residues of individual chemicals when applied once a year. A retrospective analysis of the actual amounts

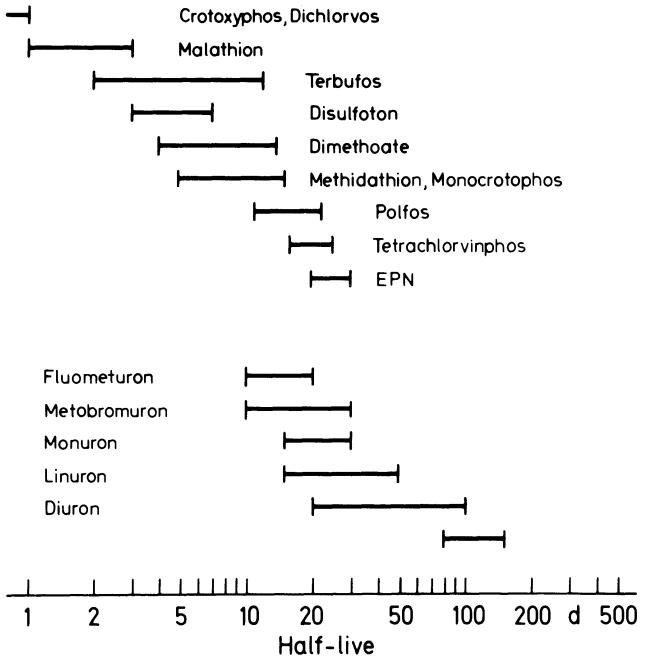

Fig. 3. Examples for ranges of half-lifes of pesticides

of pesticide residues in rural areas of the USA in 1968–73 revealed that only traces of 27 different pesticides, mostly chlorinated hydrocarbons, could be detected[3]. Considering the relatively high application level at that time and the resulting low residue values (ranging from 0.002 to 0.015 $\mu g\, g^{-1}$) the *chronic* impact on non-target organisms must be assumed to be small. This, however, does not exclude the possibility that the *acute* impact immediately following application can still be significant.

Distribution

The movement of a chemical on and in soil is determined by physical and chemical properties. *Horizontal* distribution of pesticides on the soil surface through run-off or wind erosion usually affects less than 0.5% of the applied dose, except for situations of heavy rainfall 1 to 2 weeks after application, wettable powders with low water solubility are used, or in cases of highly water soluble products ($\geqslant 10\, \mu g\, g^{-1}$) which dissolve directly in the run-off water[11].

The *vertical* distribution in the surface layers of soils is usually documented in leaching experiments. A low mobility is requested in order to prevent the ground water from being contaminated. It is not surprising that chemicals with low water solubility do not percolate

in a physical and chemical environment with high capacities for filtering, adsorbing or degrading them. But this apparent advantage of limited vertical distribution in the field leads to an accumulation in a shallow layer of top soil. The consequences for microorganisms are first of all a higher probability for intimate contacts with potentially toxic pesticide molecules, secondly a longer lasting flux of chemicals from binding or sorption sites into soil solution, and thirdly the exclusion of those microorganisms dwelling in deeper layers from pesticide degradation. Since these factors operate against efficient microbial performance, the frequently recorded discrepancies between field and laboratory tests could possibly be explained by the restricted vertical distribution under field conditions.

It is still not known to what extent a postulated lasting flux of 'bound residues' into the soil solution might affect sensitive microbiological processes and be responsible for what has repeatedly been described as 'persistent effect'. What appears like a theoretical speculation at this time has one practical consequence: the necessity of monitoring periods long enough to eventually detect the onset of chronical effects.

Toxicity

The principles of classical toxicology could probably be applied to characterize effective concentrations of pesticides on biological processes in soils. It is, however, of little value to know precisely the dose at which, *e.g.* 50% of an essential function is eliminated, rather than knowing that a chemical in ineffective within a presumed range of environmental concentrations. In the same context the concept of single species testing, which still dominates aquatic ecotoxicology, should only in exceptional cases be applied to soil problems. It is often not fully appreciated that in many respects each soil sample represents a miniaturized and integrated 'ecosystem' in itself.

In selecting appropriate test parameters all serious efforts are directed toward systems that are linked to soil productivity or, in more general terms, soil fertility. Theoretically there is no particular array of tests but doubless three of the more essential microbiological functions are degradation of organic matter (mineralization, transformation), accumulation of bioelements (N-fixation, P-accumulation, storage in living biomass) and contributions towards a stabilization of terrestrial eco-subsystems.

In an *empirical* analysis of the actual responses of 25 microbiological processes to 71 different pesticides in 734 experiments we found:

Table 1. Environmental stress imposed on microbial functions in soil (from Domsch *et al.*[6])

Environmental stress	Causal connection	Effects
Temperature	Transition from optimal range (mesophilics) to higher or lower temperatures	Frequent depression of functions (and populations) of > 50% up to 99% possible
Water potential	Transition to low water availability; lack of available water, high salinity	Frequent depressions of > 50%, up to 100% possible
Soil atmosphere	Transition to anaerobic conditions by compaction, flooding	Depressions of 50% observed, up to 99% possible
Energy supply	Decreased supply of reduced carbon	Frequent depressions of < 50%, up to 90% possible
Natural inhibitors	Input from secondary metabolic activities of other organisms	Depressions up to > 50% possible

acid phosphatase activity, organic matter degradation, and nitrification

to be sensitive indicators while

denitrification, urease activity, and non-symbiotic N_2-fixation

proved to be rather insensitive. So far ammonification, CO_2-production, O_2-uptake and dehydrogenase activity take an intermediate position. Up to now other functions are not yet well enough documented for final judgement. It is probably premature to link the sensitivity of a specific process with certain types of pesticides but it might be mentioned that we find phosphatase particularly responsive to organic phosphorus insecticides, and nitrification to fumigants and fungicides.

The accumulated information leaves no doubt that a *toxic* agent reaching the soil can effect changes within the system. However, the ecological *significance* of an induced change cannot be estimated without taking the natural fluctuations within the system into account. Some examples of negative effects which are caused by natural events are listed in Table 1. This shows that microorganisms may suffer from environmental stress but the affected functions usually resume normality soon after the end of a stress situation. Frequently, measured recovery periods are in the range of 20–30 days[6].

Evidently the negative effects under consideration can be characterized and measured in two ways: by the *magnitude* and by the *duration* of growth depressions. Surprisingly almost all microbial responses can be described by only four basic types, including both *reversible* and *persistent* reactions as defined by Domsch *et al.*[6]

In both ways the time factor is the most essential critierion. From an ecological point of view the duration of a depression ranks higher than

Table 2. Distribution of cases of reversible and persistent effects within three categories of ecological impact (data from Domsch *et al.*[6] and recent evaluations)

Number of cases* analysed	Response type	Ecological impact			Total response
		Negligible	Tolerable	Critical	
1.630	Reversible	240 (15%)	34 (2%)	15 (1%)	289 (18%)
1.630	Persistent	222 (13%)	388 (24%)	37 (2%)	647 (39%)

* Each combination of a microbiological test and individual pesticide is regarded as a single 'case'.

its magnitude. For reversible reactions recovery (delay) periods of 30 days can be regarded as a more or less natural event so Domsch *et al.*[6] proposed to group delay periods of

$\leqslant 30$ days as negligible

31–60 days as tolerable, and

> 60 days as critical.

Some justification can be made for extending the tolerable delay period to 60 days because man-made agroecosystems are not representative of 'natural' ecosystems and are therefore nor appropriate for enforcing rigid ecological principles.

Based on the above criteria an evaluation of the available data on *reversible* pesticide side-effects shows a distribution which is contained in Table 2.

The distribution shows that the test systems in present use are sufficiently sensitive. In the majority of cases no measurable effect has been recorded. Critical responses are rare (1%): they are mainly concerned with nitrification and the effects on this process of fumigants such as fumazone, chloropicrin and ethylene dibromide.

Much more serious than delay in process recovery are all those effects which appear to persist over a considerable monitoring period[6]. If a microbial process does not return to normality and/or recovery remains incomplete, then the difference between a pesticide treatment and a control is a suitable measure. This difference is expressed as a percentage.

The distribution of cases so far observed and evaluated is contained in Table 2. Again more than 50% of all cases show no reaction. Of the remainder only 2% indicate a critical response. As far as processes are affected it is again the nitrification, cellulose degradation and O_2-uptake are sensitive, particularly to fumigants, including the ones mentioned above and methyl bromide and allyl alcohol.

The nature of true persistent effects is not yet fully understood. It is possible that the energy for cell maintenance is limited thus preventing complete recovery.

In conclusion it can be stated, that the application of pesticides in recommended rates has not resulted in harmful effects on soil microbiological processes, if – as an exception – the use of biocidal fumigants is regarded as a calculated and not an injurious risk.

Heavy metals

Input

Heavy metals reach the soil by direct application, direct deposition of emissions and indirectly by contaminated litter. Naturally occurring or geogenic heavy metals are normally not immediately available to microorganisms. The ones released by human activities, however, may be bio-available to a much greater extent.

Microorganisms play an active role in the transfer of heavy metal species from organic matter and/or crystalline minerals and precipitates into the soil solution. During the decomposition of organic substrates the amount of heavy metals released is proportional to the mineralization rate.

Toxicity

A toxicological approach would advise the elaboration of dose-effect relationships. In soils, however, an effective heavy metal 'dose' can only roughly be estimated. It is mainly the chemical environment which determines the equilibrium between bioeffective heavy metal ions in solution and the total amount contained in soil. It is for this reason that the determination of so-called 'maximum tolerable concentrations' after a stepwise experimental soil enrichment with heavy metals is not a meaningful approach. Furthermore, negative influences can practically only be expressed within ranges. Within these ranges soil pH, base saturation, amount and quality of soil organic matter, clay mineral content and interactions with other soil constituents are of special importance.

It should also be recognized that toxic agents come into contact with *populations* of microorganisms. The sensitivities within a given population follow a statistical distribution and under continuing selection pressure heavy metal tolerant and/or resistent organisms will emerge. Counting viable propagules in heavy metal containing soils might therefore produce misleading results. On the other hand, the measurement of a microbial function will not indicate population shifts as long as the process itself is not negatively influenced.

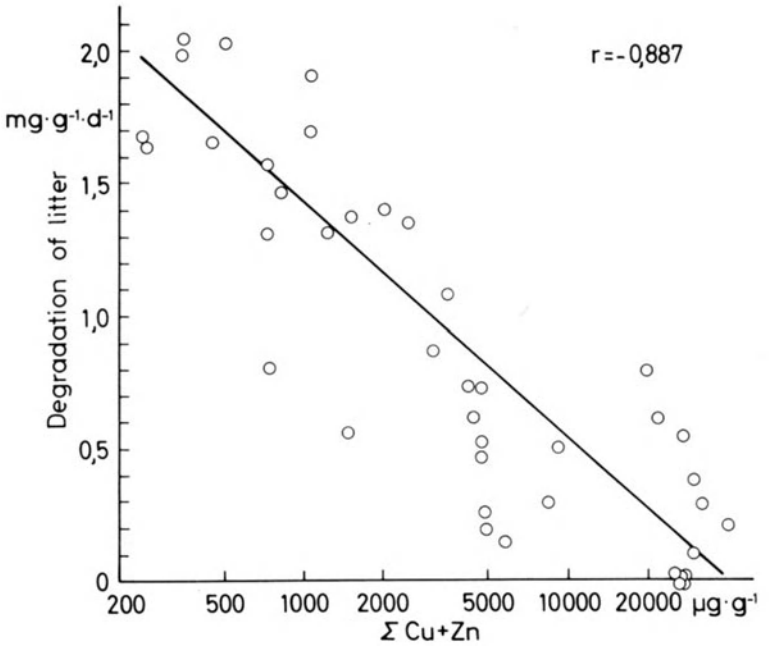

Fig. 4. Relationship between Cu + Zn contents and degradation of forest litter (from Tyler 1976)

As mentioned earlier litter degradation is a simple and ecologically important measure of a multitude of integrated microbial functions. Extremely instructive data have been produced from pollution gradients in the field. An example taken from a study of forest litter degration[9] is reproduced in Fig. 4. Admittedly the Cu + Zn-contents which would reduce litter decomposition by 50% are extraordinary high but they also show without any doubt how close man-made heavy metal contents have already approached the range of critical and possibly persistent effects.

Another example is given in Fig. 5, concerning cadmium as a toxic agent and progressive litter accumulation in the presence of increasing Cd contents[5]. A specific feature of these experiments was the observation that the proportion of small organic matter particles increased with increasing Cd contents, indicating that an adapted soil fauna was still effective. This leads to the conclusion that a prospective evaluation of ecotoxicological effects may be obscured as long as the potential for restitution and repair is not yet exhausted.

Regarding the application of sewage sludge on arable land numerous attempts have been made to assess the risk. A frequent approach is:

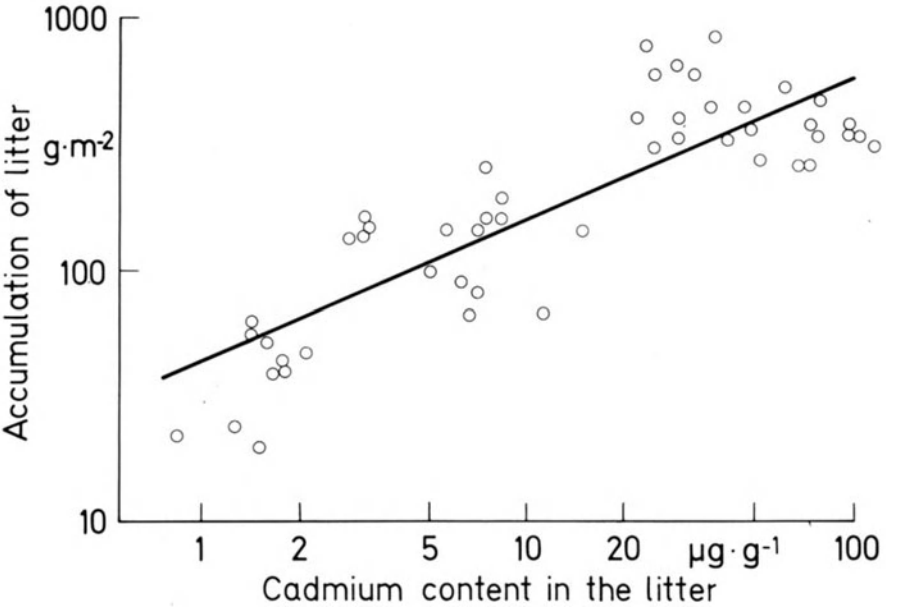

Fig. 5. Relationship between Cd contents and accumulation of litter (from Coughtrey *et al.* 1979)

determine the heavy metal content of a series of apparently non-polluted soils and establish mean values; determine the average heavy metal load in sewage and calculate a reliable mean; determine plant uptake under assumed average soil conditions and well below phytotoxic concentrations; and finally define 'tolerable' application rates per annum which are low enough to avoid a critical accumulation of heavy metals for the future.

There are indications, however, that the permissible rates may very soon result in soil contents which would effectively inhibit catabolic reactions in soil: examples for Cd, Cu and Zn are given in Fig. 6.

The nitrification process is more sensitive to the lower pH range (pH 5.4–5.8) and not inhibited at Cu, Zn and Ni contents which are considered 'tolerable'. Only Ag and Hg show a more pronounced inhibitory effect; the pH shift during the nitrification process has no measureable effect on heavy metal mobilization[4].

The influence of heavy metals on enzymes has frequently been investigated. Three series with a comparable set of heavy metals and enzymes acting on P-, S- or N-cycles are summarized in Table 3. The effects will be modified by local soil conditions; they will be aggravated under acid conditions.

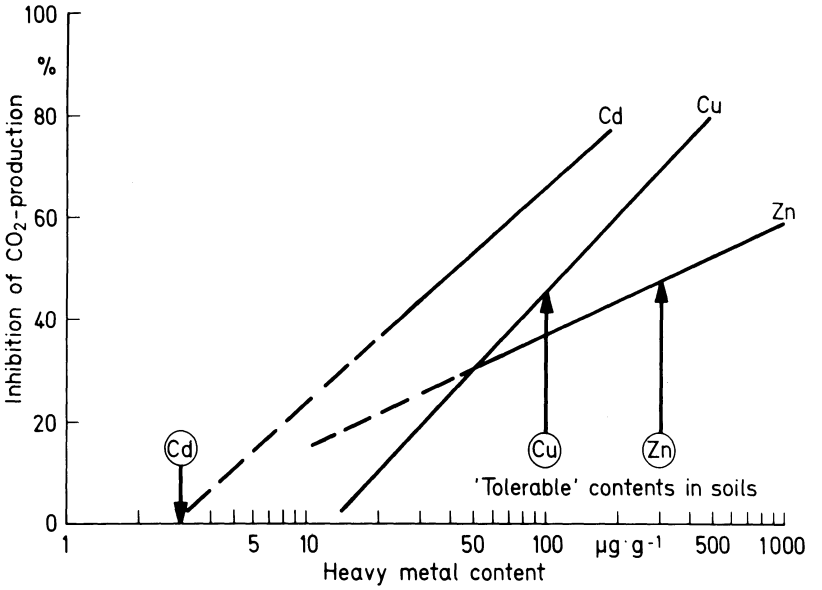

Fig. 6. Relationship between contents of Cd, Cu and Zn and CO_2-production of soil samples (from various sources)

Table 3. Inhibition of enzymes in soil; means from 2, 4 and 6 soils, resp. (from Tyler[10]; Al-Khafaji and Tabatabai[2] and Tabatabai[8])

Heavy metal (valence)	Percent enzyme inhibition in soil		
	Phosphatase (at 10 µmol [SM] g^{-1})	Arylsulfatase (at 25 µmol [SM] g^{-1})	Urease (at [5] µmol [SM] g^{-1})
Ag (I)	39	96	93
Cd (II)	6	37	54
Hg (II)	10	94	88
Cr (III)	5	41	27
W (VI)	7	25	0
Cu (II)	–	24	68
Mo(VI)	59	54	12
Sn (II)	–	37	26
Zn (II)	5	27	39
V (IV)	–	88	23

Conclusions and outlook

In general there are few signs of biological processes in soil being seriously influenced by the intentional or non-intentional application of chemicals. Individual *chemicals* are known which will significantly depress and delay biological processes; they include soil fumigants and heavy metals close to point sources. Microbial *processes* which are particularly sensitive towards chemical stressors, include phosphatase activity, organic matter degradation and nitrification.

With pesticides a reduction in sales figures is to be noticed and the recommended load per unit area is no longer increasing; the replacement of broad band pesticides by specific ones is not necessarily reflected in a shift of side-effect phenomena, but the study of side effects has partially moved from population studies to possible interferences with bioelement cycles to enzyme inhibitions.

For both pesticides and heavy metals it can be stated that the frequently hypothesized synergistic and/or accumulative actions have not yet been substantiated, interactions between soil organisms or sequences of processes are still only rarely encountered. In contrast to the increasing understanding of the microbial *degradation* of organic xenobiotics the development of even simple *models* for soils is still lacking, but at least ecological concepts are being developed in order to improve the evaluation of stress phenomena.

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Microbial populations in trifluralin-treated soil

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Key words Actinomycetes Bacteria Chernozemic soil Fungi Rhizosphere Trifluralin herbicide Wheat

Summary This study examined the effects of trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine), a soil incorporated herbicide, on soil microflora both in the general soil environment and in the rhizosphere of trifluralin damaged wheat roots. Two Dark Brown Chernozemic soils were treated with various trifluralin rates in the growth chamber and wheat [*Triticum aestivum* L. 'Neepawa'] was seeded. Trifluralin generally had no effect on fungi, bacteria, or actinomycete populations in either the general soil or in the rhizosphere. CO_2 evolution was unchanged when trifluralin was added to the soil. In wheat plots, at two field locations, there were no significant effects of trifluralin ($1.0\,kg\,ha^{-1}$) on soil fungi, bacteria, actinomycete, denitrifying bacteria, and nitrifying Nitrobacter propulations. A pure culture study with 42 soil microorganisms showed that many isolates were inhibited at 400 to 100,000 $\mu g\,g^{-1}$ but not at concentrations $< 16\,\mu g\,g^{-1}$. Similar data were obtained from tests on four different soils. These studies indicate that trifluralin is unlikely to cause changes in the numbers of soil microorganisms when used at recommended levels.

Introduction

Trifluralin is used for weed control in a variety of crops from canola (*Brassica napus*) to wheat (*Triticum aestivum*). It is soil incorporated and will interact with non-target soil organisms. Carter and Camper[5] reported that repeated applications of trifluralin reduced bacterial populations in soil but increased fungal and actinomycete populations. In contrast others have found that trifluralin appears to inhibit some fungi, *i.e.*, *Rhizoctonia solani*, *Fusarium oxysporum*[8], *Sclerotium rolfsii*[18]. Other studies have detected either no effect or else a short term reduction of microbial groups in trifluralin treated soils[10,12]. Soil respiration and enzymatic activity also exhibit variable responses towards trifluralin[6,7,13,14,20].

This study examined the response of microbial populations in the rhizosphere of wheat roots and surrounding soil after trifluralin incorporation. Studies were conducted on field plots and in a growth

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chamber. Data are also presented on the effects of trifluralin on the growth of soil microorganisms in pure culture.

Materials and methods

Field study

Field plots were established at the University of Saskatchewan Kernen Research Farm (Sutherland clay loam) and at the Agriculture Canada Experimental Farm at Scott, Saskatchewan (Scott loam) to examine the response of wheat [*Triticum aestivum* L. 'Neepawa'] to soil incorporated trifluralin (Treflan E.C.). Bulk surface soil samples were taken throughout the growing season from the control and trifluralin treated (1.12 kg ha^{-1}, Kernen; 1.0 kg ha^{-1}, Scott) plots at various time intervals. Samples were analyzed for population estimates of fungi, actinomycetes, and bacteria, including nitrifiers and denitrifiers.

Growth chamber and laboratory study

Experiment 1. Field rate concentrations Two Dark Brown Chernozemic soils, an Asquith and a Bradwell, were used. Commercial trifluralin (Treflan E.C.) was added to soil samples to provide concentrations of 0.0, 0.09 and 0.18 μg g^{-1} or 0.0, 0.4, and 0.8 μg g^{-1} in the Asquith and Bradwell soils respectively. The rates were determined in another experiment such that trifluralin injury to wheat seedlings was sublethal at the highest concentration. The soils were brought to field moisture capacity, thoroughly mixed, and allowed to equilibrate for 12 hours before planting.

Black plastic tubes, 0.051 m inside diameter by 0.4 m long, containing 1.5 kg of soil, were used as plant growth containers. Three wheat caryopses were planted per tube and the seedlings thinned to one plant per tube. Each treatment was replicated three times, randomly placed in a growth chamber and soil kept at field moisture capacity. Nine days after seedling soil cores were removed from the tubes and the root systems recovered. Soil clinging to the roots was designated the rhizosphere soil and the remaining soil designated the bulk soil. Both sets of soils were analyzed for population estimates of fungi, actinomycetes, and bacteria.

Experiment 2. Microbial CO_2 respiration Asquith and Bradwell soils were prepared for respiration studies with and without added coarsely ground wheat straw (1650 mg kg^{-1}, based on 3300 kg straw ha^{-1}) and with and without trifluralin at concentrations of 0.0, 0.18, 0.36, and 3.6 μg g^{-1}, or 0.0, 0.8, 1.6 and 16 μg g^{-1} for the two soils respectively. The treated soils were brought to field capacity, thoroughly mixed, and 0.1 kg (oven dry basis) placed into plastic vials and incubated in 2.26 l screw cap glass jars. A plastic vial containing 30 ml of 0.5 *M* NaOH solution was placed in each jar as a CO_2 trap and a few ml of water were added to the jars to create a saturated atmosphere. The jars were sealed and kept in the dark at a day temperature of 295 K and a night temperature of 290 K. The jars were recharged with air and fresh NaOH every week when the NaOH samples were titrated with 0.1 *M* HCl using the carbonic anhydrase method[21]. All treatments were replicated twice.

Microbial analysis

The spread-plate technique was used for total counts of fungi, bacteria, and actinomycetes. The media employed were: Difco Czapek-Dox Agar (pH 3.5) for fungi (Difco Laboratories, Detroit, Mich.), 0.3% Trypticase-Soy-Agar for bacteria[15], and Difco Actinomycete Isolation Agar with added actidione (50 mg L^{-1}) and tetracycline (5.0 mg L^{-1}) for actinomycetes. All media were incubated at 298 K and counts taken after four to nine days.

Nitrifying (*Nitrobacter* sp.) and denitrifying bacteria were determined using the most probable number (MPN) technique[1,2]. Nitrite nutrient broth[22] in microtiter plates was used for the dentrifying bacteria. These plates were incubated at room temperature for 7 days in a vacuum dessicator filled with 100% He[19]. The absence of nitrite upon the addition of N-1-Napthylethylene diamine Dihydrochlorite (NEDD) reagent[22] indicated the presence of

Table 1. Effect of trifluralin on growth of various soil microorganisms in pure culture

Organism	Growth[1] medium	Trifluralin concentration[2] ($\mu g\ g^{-1}$) 400	4,000	16,000	25,000	50,000	100,000
Fungi							
Absidia glauca	PD	–[3]	–	+	NT[4]	NT	NT
Aspergillus fumigatus	PD	–	–	–	–	+	++
Chrysosporium pannorum	PD	–	–	–	+	+	++
Chrysosporium sp.	PD	–	–	–	+	+	++
Cladosporium herbarum	PD	–	–	–	+	+	+
Fusarium culmorum	PD	–	–	+	+	+	++
Fusarium epishaeria	PD	–	–	–	–	+	+
Fusarium roseum	PD	–	–	–	+	+	++
Gliocladium roseum	PD	–	–	–	–	+	++
Mortierella isabellina	PD	–	–	–	–	–	–
Mortierella nana	PD	–	–	+	++	++++	+++++
Mucor silvaticus	PD	–	–	–	+	+	++
Paecilomyces carneus	PD	–	–	–	+	+	+
Paecilomyces marquandii	PD	–	–	+	+	+	++
Penicillium frequentans	PD	–	–	–	–	–	–
Penicillium nalgiouensis	PD	–	–	–	–	–	–
Sporotrichum carnis	PD	–	–	+	+	++	++++
Trichorderma hamatum	PD	–	–	+	+	+	++
Trichoderma viride	PD	–	–	–	–	+	++
Zygorrhynchus vuilleminii	PD	–	+	++	+	+++	+++
Bacteria							
Agromyces ramosus ATCC 25173	HI	+++	+++++	+++++	+++++	+++++	+++++
Arthrobacter globiformis ATCC 8010	NA	–	+	++	NT	NT	NT
Arthrobacter oxydans ATCC 14358	NA	–	+	+++	NT	NT	NT
Azospirillum brasilense ATCC 29145	CCM	–	–	–	–	–	–
Azotobacter chroococcum ATCC 9043	BA	–	–	–	–	–	–
Azotobacter vinelandii ATCC 12837	BA	–	–	–	–	–	–
Bacillus megaterium	NA	+	+	+	++++	+++++	+++++
Enterobacter aerogenes	NA	–	–	–	–	–	+
Micrococcus flavis	NA	–	–	+	NT	NT	NT
Pseudomonas aeruginosa	NA	–	–	–	–	–	–
Pseudomonas fluorescens	NA	–	–	–	–	–	–
Rhizobium leguminosarum 128C30	TY	–	–	–	+	++	+++
Rhizobium leguminosarum 128C52	TY	–	–	–	+	++	+++
Rhizobium leguminosarum 128C79	TY	–	–	–	+	+	++
Rhizobium meliloti NRG185	TY	–	+	++	+++	+++	+++
Rhizobium meliloti NRG9930	TY	–	–	–	++	+	+

Table 1 (*Continued*) . . .

Organism	Growth[1] medium	Trifluralin concentration[2] ($\mu g\,g^{-1}$) 400	4,000	16,000	25,000	50,000	100,000
Rhizobium trifolii 14480	TY	–	–	–	–	+	++
Rhizobium trifolii TAI	TY	+	+	++	++	++	+++
Actinomycetes							
Streptomyces sp.	NA	–	+	++	++	+++	+++
Streptomyces sp. A-12	NA	–	+	+	NT	NT	NT
Steptomyces sp. A-16	NA	–	–	+	++	+++++	+++++
Streptomyces sp. S-34	NA	–	–	+	NT	NT	NT

[1] PD = Potato Dextrose Agar; HI = Heart Infusion Agar; NA = Nutrient Agar; CCM = Comined Carbon Medium (Rennie, 1981); Ba = Burks Agar (Burk, 1930); TY = Tryptone Yeast Extract Agar (Hirsch *et al.*, 1980).

[2] Trifluralin concentrations of 0.4–16 ppm tested but no effect observed.

[3] Size of inhibition zone: –, no effect; +, > 0 to ⩽ 5 mm; ++, > 5 to ⩽ 10 mm; +++, > 10 to ⩽ 15 mm; ++++, > 15 ⩽ 20 mm; +++++, > 20 mm.

[4] Not tested.

denitrifying bacteria. Nitrobacter populations were determined after 21 days incubation at 298 K using nitrite medium in test tubes and reagents described by Alexander and Francis[3].

For all soils examined, serial 10-fold dilutions were prepared using 10 g (moist weight) subsamples of soil. Counts of fungi, bacateria and actinomycetes populations represent the average of three replicate plates for three soil sample replicates. The MPN results are the average of one MPN test per soil replicate. All microbial counts were expressed as number of organisms per gram of oven-dried soil.

Pure culture

A sensitivity test was used to assess the minimum inhibitory concentration (M.I.C.) of trifluralin on microbial growth. The soil microorganisms and media used are listed in Table 1. Prepared media plates were inoculated (*i.e.*, complete surface coverage) with a 72 hour broth stock culture of the appropriate microorganisms. Trifluralin (Treflan E.C.) was passed through a sterile 0.22 μm nucleopore filter and stock suspensions were prepared in sterile water with concentrations of 0.4, 4, 16, 400, 4000, 16000, 25000, 5000, and 100000 $\mu g\,g^{-1}$. Sterile paper discs (Schleicher & Schuell, Inc., Keene, N.H.) 12.7 mm in diameter were treated with 3 or 4 drops of the filtered trifluralin stock suspensions. Sterile distilled water was the control solution. One control disc and three trifluralin discs, each with a different concentration, were aseptically transferred onto the preinoculated surface of each plate (replicated twice) and incubated at 298 K. After 2 to 3 days of incubation, the plates were examined for zones of inhibition around each disc.

Results and discussion

Field study

The microbial population levels in the bulk soil from the control plots were very similar. For example, at the first sampling time the fungal, bacterial and actinomycete counts for the Kernen site were respectively 1.15×10^4, 9.61×10^6, and 9.21×10^5 g^{-1} soil compared to 1.39×10^4, 3.86×10^6, and 7.96×10^5 g^{-1} soil, for the Scott site. The

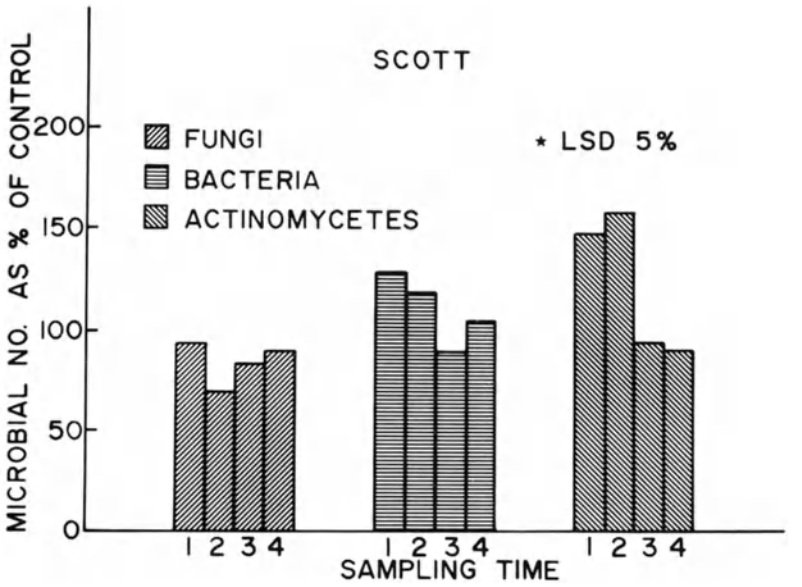

Fig. 1. Effect of trifluralin on microbial populations in the Scott bulk field soil; sampling time 1, day of herbicide incorporation; 2, two weeks; 3, six weeks; 4, ten weeks post herbicide incorporation.

nitrifying and denitrifying populations were also similar, and comparable poulation levels continued for all microbial groups throughout the entire sampling period.

Wheat plants in the trifluralin treated plots exhibited reduced plant density and signs of injury such as tip damage of primary leaves, reduced root growth, and swelling of root tips. In the Scott bulk soil, slight inhibition of fungal populations and an initial period of stimulation of bacteria and actinomycete populations occurred, but no significant trend was evident (Fig. 1). There was no trifluralin effect on nitrifying and denitrifying populations (data not presented). Rhizosphere organisms could not be assessed because of difficulty in removing the root systems from the field soil. The Kernen bulk soil data (not presented) were very similar. In order to examine the microbial populations in the rhizosphere of trifluralin-damaged wheat plants and to verify bulk field soil results, a series of growth chamber studies were initiated.

Growth chamber study

Trifluralin concentrations of 0.18 (Asquith) and 0.8 (Bradwell) $\mu g\, g^{-1}$ soil caused considerable damage to wheat seedlings similar to that described for our field study and by Olson[16]. The effects of

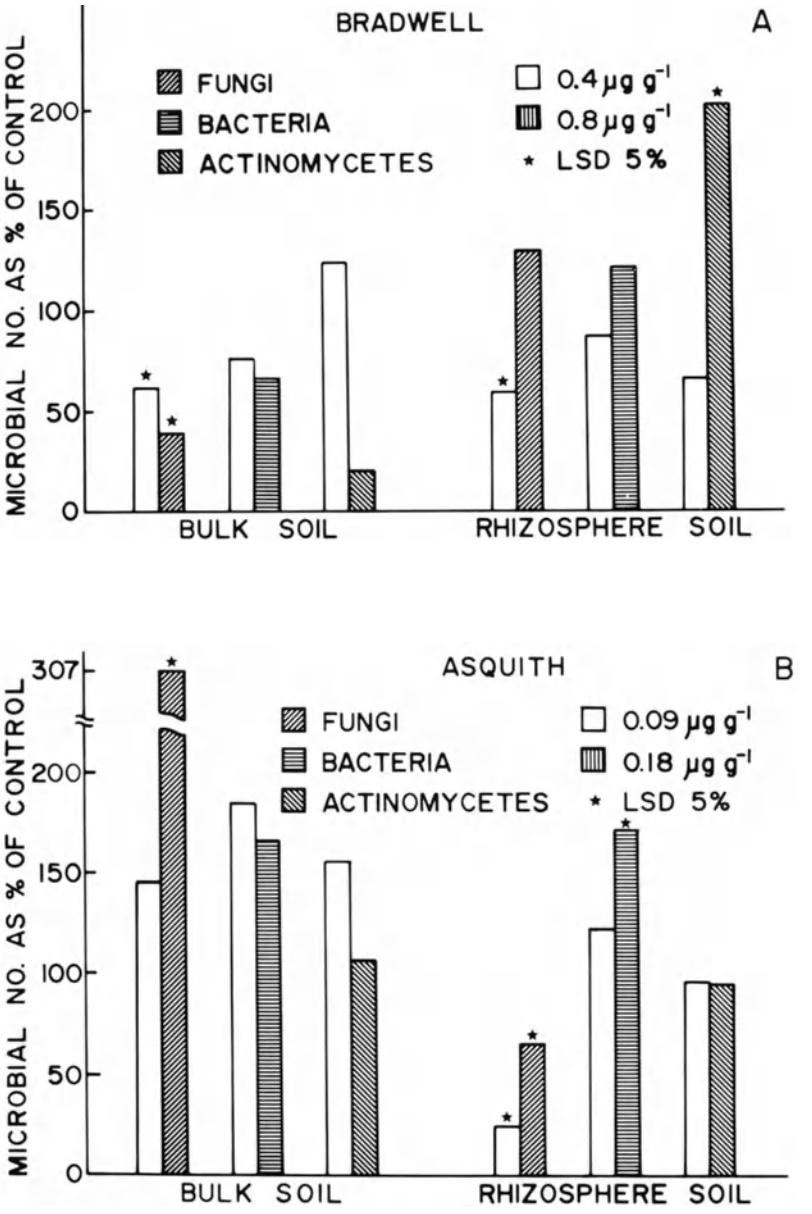

Fig. 2. Effect of trifluralin on microbial populations in bulk and rhizosphere soil of 9 day old wheat plants grown in a growth chamber.

trifluralin amendments on the microbial populations in the bulk and rhizosphere soil are shown in Fig. 2a (Bradwell) and Fig. 2b (Asquith). Fungal, bacterial, and actinomycete populations in the Asquith control

sample were 3.52×10^3, 6.81×10^6, 7.23×10^5 (bulk soil) and 2.01×10^4, 3.68×10^7, 4.69×10^6 (rhizosphere) per gram of soil, respectively, and in the Bradwell control samples, 4.26×10^4, 5.86×10^7, 1.74×10^6 (bulk soil) and 2.46×10^4, 1.32×10^8, 3.11×10^6 (rhizosphere) per gram of soil, respectively. Trifluralin affected the fungal populations in both bulk and rhizosphere fractions of each soil. Both stimulation and inhibition occurred so no clear-cut conclusion can be drawn but our results seemed to indicate that soil fungi were more sensitive to trifluralin than bacteria and actinomycetes.

To test the effects of excessive trifluralin rates on microbial populations, the growth chamber experiment was repeated but without wheat plants since the herbicide rate used ($16\,\mu g\,g^{-1}$) was lethal. The three microbial population levels were unaffected in the Asquith soil. In the Bradwell soil, however, the bacterial population decreased 60%, whereas the fungal and actinomycete populations increased 27% and 100%, respectively. Though the actinomycetes appeared to be greatly stimulated, the variability was large enough to make it non-significant.

Greaves *et al.*[9] stated that soil microbial population changes due to herbicides should be compared to the population variation that occurs under natural conditions. They proposed that a population would have to be decreased 50% to be considered ecologically significant. These guidelines should also apply to any increase in microbial population levels. Our results show most of the microbial population levels fall within these limits and nearly half that were outside these limits were statistically non-significant.

Microbial CO_2 respiration

The overall microbial activity measured by CO_2 evolution was unaffected by trifluralin ($1.6\,\mu g\,g^{-1}$) in the Bradwell soil (Fig. 3). Added wheat straw caused a large increase in CO_2 evolution but trifluralin neither stimulated nor inhibited straw decomposition. The results for the Asquith soil (not presented) were similar. The experiment was repeated $16.0\,\mu g\,g^{-1}$ trifluralin in both soils and produced similar results. Davies and Marsh[7] observed no effect of trifluralin ($100\,\mu g\,g^{-1}$) on CO_2 evolution and N-mineralization.

Pure culture studies

Though the field rates and higher trifluralin concentrations in the previous experiments did not significantly alter microbial populations, it is important to determine at what concentrations trifluralin would affect pure cultures grown under laboratory conditions (Table 1). None of the organisms tested were sensitive to levels of trifluralin below

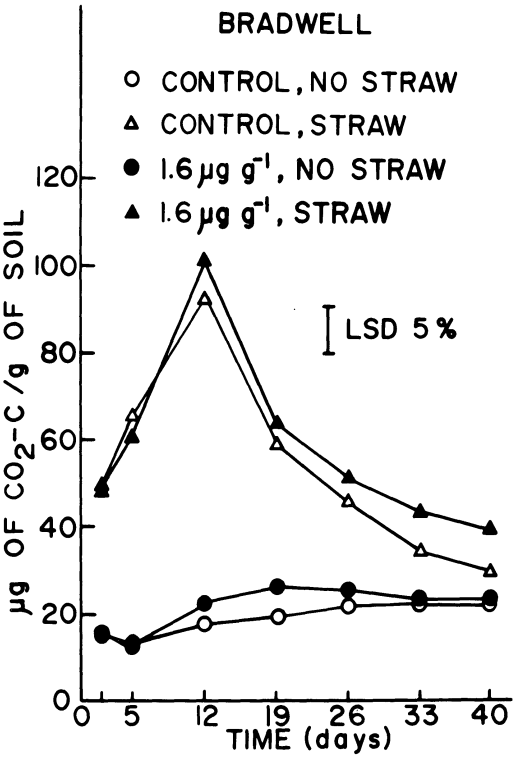

Fig. 3. Effect of trifluralin on microbial respiration in straw amended and non-amended soil.

16 $\mu g\,g^{-1}$. Most fungal isolates were unaffected at concentrations < 16,000 $\mu g\,g^{-1}$ but were inhibited by concentrations in excess of 25,000 $\mu g\,g^{-1}$. Most of the bacteria and the four actinomycetes tested were inhibited at lower trifluralin concentrations, though generally inhibition was slight at concentrations less than 4000 $\mu g\,g^{-1}$.

The soil microflora were not significantly affected by trifluralin when it was used at field stress (0.6 to 0.8 $kg\,ha^{-1}$, approximately 1.5 to 2.0 $\mu g\,g^{-1}$ of soil). Nevertheless, the trifluralin concentration at the surface of carrier particles in the granular formulation (5% trifluralin by weight) is approximately 70,000 $\mu g\,g^{-1}$ and until the herbicide diffuses from the carrier material under moist conditions soil microbes around granules might be inhibited as observed in pure cultures.

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Chemical disinfestation and metabolic integrity of soil

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Key words 1,3-Dichloropropene Ethoprophos Fumigation Herbicide degradation Metham-sodium Nematicides Organic matter mineralisation

Summary Field disinfestation in autumn with normal or increased dosages of 1,3-dichloropropene, metham-sodium or chloroform, and in spring with ethoprophos, did not, or hardly, affect the degradation *in situ* of some herbicides applied in spring. However, during laboratory incubation of samples from the disinfested plots, sometimes decreased herbicide degradation rates or increased lag phases were found.

The top few centimetres of the field soil, on which the herbicides were sprayed, apparently largely escaped fumigation. Accordingly, effects of fumigation on herbicide degradation, organic matter and N metabolism were stronger in samples from the 10–20 cm layer than in samples from 0–10 cm.

In the laboratory fumigation of soil samples with $CHCl_3$ was much more drastic in inhibiting herbicide degradation, N mineralisation and nitrification; inoculation with 10% fresh soil, however, greatly accelerated the recovery of these processes. Therefore, in practice prolonged or drastic effects of chemical soil disinfestation on metabolic integrity of the soil are not to be expected.

Introduction

Soil disinfestation with fumigants, particularly Telone (1,3 dichloropropene about 150 kg/ha) or granular nematicides (3–10 kg/ha) is a common practice in potato growing areas in The Netherlands. Fumigants not only kill nematodes but can also affect certain soil microbial activities[1,8]. The question arose whether soil fumigation affects the microbial degradation of herbicides applied afterwards, particularly when the degradation is due to a limited number of species, as may be the case for chloridazon. Between autumn fumigation and spring application of herbicides, however, the soil microflora has time to recover, since fumigants usually disappear rapidly. Granular nematicides are worked into the 0–10 cm layer in spring at about the same time as some herbicides, so a real possibility of interaction exists.

Mineralisation of organic matter is a common function of the soil microflora. Soil disinfestation kills part of the microflora, but surviving microbes usually take over and rapidly restore the mineralisation rate. Nitrification is more vulnerable to disturbances by pesticides. We investigated the effects of disinfestation on these processes and on herbicide degradation.

Table 1. Characteristics of the soils used in experiments 1–4; 0–20 cm layer

Location	Soil type	Texture	Organic matter (%)	Clay < 2 μm (%)	Silt 2–16 μm (%)	$CaCO_3$ (%)	pH (1 M KCl) 1:5
1. Marknesse	Fluvisol	calc. SL	2.4	18	14	8	7.5
2. Haren	Podzol	S	3.4	4	3	–	4.7

Field and laboratory techniques, established in preliminary experiments in 1979/80, were subsequently used in replicated trials in 1980/83.

Materials and methods

Because soil fumigation in the field cannot be imitated accurately in the laboratory, field trials were performed principally on two soil types: an acid sandy soil and a calcareous sandy loam soil, a representative of the area (140,000 ha) that is periodically disinfested (Table 1). Plots of 9 or 25 m^2 were fumigated using a fumigant-injector (Shell) at 18 cm depth in 16 or 36 spots per m^2. The granular nematicide Mocap (ethoprophos) was evenly applied on the soil surface and worked into the top 10 cm by rotavation. The separate plots were carefully hand-sprayed with formulated herbicides or mixtures thereof. Application rates ranged from 2.4 to 3.2 kg active ingredient (a.i.)/ha, or 1.8 to 2.6 mg/kg soil (0–10 cm layer) with a bulk density of 1.25 g/cm^3. Herbicides were applied in spring, except in experiments 3 and 4b in autumn. Cropping with sugar beet was preferred to cropping with potatoes, as it permits easy soil sampling. In experiment 2 the soil microflora were stimulated before fumigation by adding dried and ground cow manure enriched with urea (6000 + 130 kg/ha). To suppress volatilisation of fumigants in experiments 2 and 3, the plots were covered with plastic sheets for 8 to 16 days. The sheets were pervious to oxygen, but as appeared later, also to fumigants.

In addition, larger experimental plots that had been fumigated or treated with granular nematicides for several years with normal field equipment were monitored for herbicide residues (experiment 5). Samples of the 0–20 cm layer were taken from sugar beet and potato plots with different crop rotations.

Analytical procedures

For laboratory degradation experiments, soil samples were taken from disinfested and from control plots (0–10 cm layer); after sieving (2.5 mm) and partially air-drying they were homogeneously mixed with the same herbicides as were applied, after sampling, in the fields. Application rate was 5 mg/kg soil. Duplicate samples, containing 100 g dry matter, were brought to *ca.* 50% water holding capacity and incubated in closed 500-ml bottles at 20°C in the dark. Herbicide degradation was monitored by analysis of 5 or 10 g subsamples at 5 to 10 occasions. When half of each sample was used up, duplicates were combined to allow further sub-sampling. Some soil samples from control plots were fumigated for 24 hours with $CHCl_3$ (Jenkinson and Powlson[2]), evacuated to remove its residues and then treated with herbicides, followed by incubation (experiment 4), as a comparison for field fumigation. In some samples the soil microflora were stimulated by mixing 0.5% ground lucerne with the soil and incubating for one week before fumigation and herbicide addition, in order to test the hypothesis that a metabolically active microflora is more vulnerable to fumigation.

Residues of fumigants and of ethoprophos were extracted with hexane or ethylacetate and analysed using gas chromatography with electron capture or flame-photometric detection[4,6,7]. Herbicide residues were extracted with methanol–water 60/40 v/v, followed by reversed phase HPLC with UV-detection at properly selected wavelengths[3].

For soil respiration and N conversion studies, as reliable methods for measuring these in the field were not available, samples were taken to the laboratory immediately after removal of the plastic sheets, for incubation at 20°C.

N mineralisation was measured in soil samples with and without lucerne after 6 and 12 weeks.

Nitrification of added $(NH_4)_2SO_4$, 100 mg/kg as N for soil 1 and 25 mg/kg as N for soil 2, was determined after 2 and 6 weeks as NH_4-N and NO_3-N in 1 *M* NaCl extracts.

Urease activity was measured after 0, 6 and 12 weeks, as urea hydrolysed in 24 h (soil 1) or 16 h (soil 2) at 29°C without buffer; urea was extracted with 2 *M* KCl and determined colorimetrically[9].

Oxygen consumption (B.O.D.) was measured for 2 weeks in 400 g samples with 0.5% ground lucerne added, using electrodes developed in our institute to measure partial O_2 pressure of the air above the samples and regularly renewing the air.

Populations of free-living nematodes were counted by means of the floatation method of Oostenbrink[5]; numbers of bacteria were determined by spreading 10^{-4} to 10^{-7} soil dilutions on soil extract agar and incubation for 7 days at 22°C.

Table 2. Chemical disinfection and metabolic integrity of soil: summary of experiments

Number and year of experiment	Disinfestants used in field plots	Dosage time* and rate 1/ha or kg/ha	Herbicides used in field and/or laboratory	Number of plots per soil
Short-term experiments with soils 1 and 2				
Key T = Telone II : 92% 1,3-dichloropropene			C = Chloridazon	
Ma = Monam : 510 g/l metham sodium			D = Dinoseb	
Mc = Mocap 20G : 20% ethoprophos			L = Linuron	
Cf = Chloroform : $CHCl_3$			ML = Monolinuron	
			P = Propham	
1. 1980/'81	T	A 150	Field and Lab: C + P (both soils)	4
	Ma	A 300	ML + L (soil 1)	4
	Mc	S 50	D (soil 2)	4
	Control	–		4
2. 1982	Cf	S 150, 300 500, 1200	Field and Lab: C + P	16
	Control	–		4
3. 1982	Cf	A 600, 1200	Field and Lab:	6
	T	A 600, 1200	C + P	6
	Control	–		4
4. 1982		saturated		*pots*
a	Cf (*Lab*)	S vapour	Lab:	16
b	Cf (*Lab*)	A 24 hours	C + P	12
Long-term experiments on several other sandy soils				Number of plots (soils)
5. 1980/'82	T	A variable**	Field:	106 (5)
	Ma	A variable**	C, D, ML, P Phenmedipham	66 (4)
	Control			20 (4)

* Time of application; A = autumn and S = spring.

** From a normal dosage every 4th year to a double dosage annually.

Brief elucidation of the experiments (Table 2)

In experiments 1, 2 and 3, herbicide degradation rates were determined both in the field and in the laboratory, although disinfestation was applied only in the field. In experiment 2 we tried to create strong inhibitions and also looked for the effect of stimulating the soil microflora. Because the effect of $CHCl_3$ fell short of our expectations, in experiment 3 we only repeated the highest dosages and compared their effect with the same high dosages of Telone II. The latter completely killed nematodes and was more persistent in the soil than $CHCl_3$. Crop residues of sugar beet in autumn served as a source of microbial stimulation. In this experiment, samples from the 0–10 and 10–20 cm layers were separately incubated in the laboratory, because fumigation was suspected to be less efficient in the upper layer. Comparison of results of experiments 2 and 3 might show a different vulnerability of the soil microflora in spring and in autumn samples. In experiment 4, $CHCl_3$ fumigation of soil samples in the laboratory should give a maximum effect on the microflora, because escape of the fumigant was prevented. Recovery from fumigation was studied by adding 10% fresh soil after removal of $CHCl_3$, imitating a field situation where parts of the soil escape fumigation. Samples fumigated with $CHCl_3$ in the laboratory were tested immediately after removal of the $CHCl_3$ residues. Experiment 5 might demonstrate long-term effects of fumigation.

Results and discussion

Effects on herbicide degradation

Fig. 1 shows the degradation rates of herbicides in disinfested soils of experiment 1. In 13 of 42 cases slower herbicide degradation rates were found in samples from disinfested plots; in 10 after spring application of Mocap, in the other 3 after autumn fumigation with Telone II. Except for chloridazon in one of the Telone II plots (1d) disinfestants did not increase herbicide degradation rates. Conversely, the loam soil plots treated with Mocap showed a delay in degradation of monolinuron and linuron of about 2 weeks (1e and f). The presence of ethoprophos residues, 3.5 and 4.5 mg/kg soil 1 and 2, resp., at the start of the laboratory incubation may be the explanation. Granulated ethoprophos and the herbicides were more homogeneously distributed in the samples prepared for incubation than when applied in the field. Herbicide concentrations in the laboratory and in the field are only seemingly different. Herbicide residues in the field are given as mg/kg for the 0–10 cm layer that was sampled, but their distribution was mainly limited to the 0–5 cm layer. Residues from autumn fumigation were not detected at the time of herbicide application and the microflora had some months to recover.

$CHCl_3$ field fumigation in spring (experiment 2) did not affect the degradation rates of chloridazon and propham, either in the field or in the laboratory. Although the plots were covered with plastic, $CHCl_3$ had disappeared too rapidly from the soil. Herbicide residues were not significantly different in the manure treated plots.

Even $CHCl_3$ fumigation in the laboratory of soil samples taken in spring (experiment 4) had little or no effect on herbicide degradation.

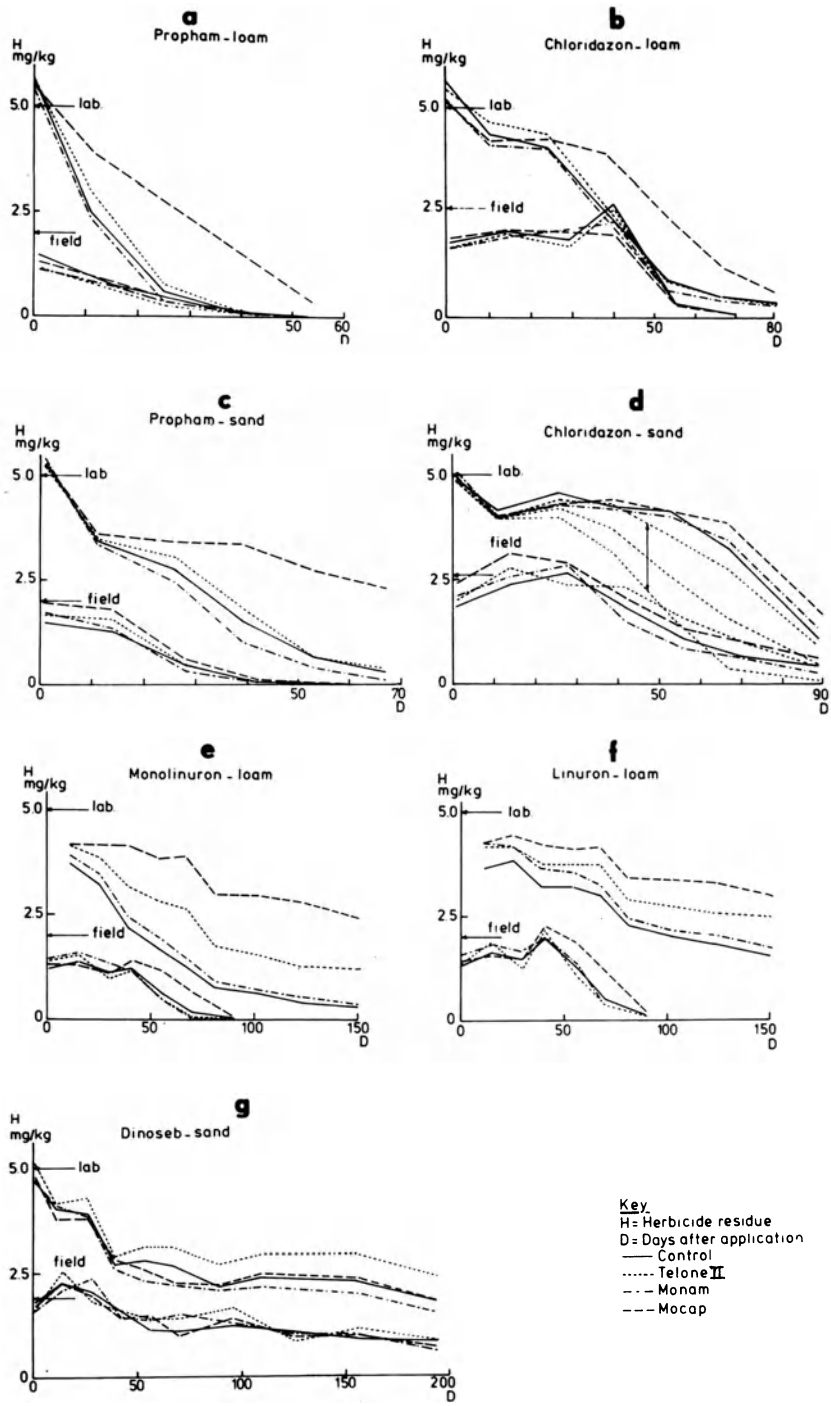

Fig. 1. Degradation of herbicides in disinfested field plots and during incubation of samples from these plots; experiment 1

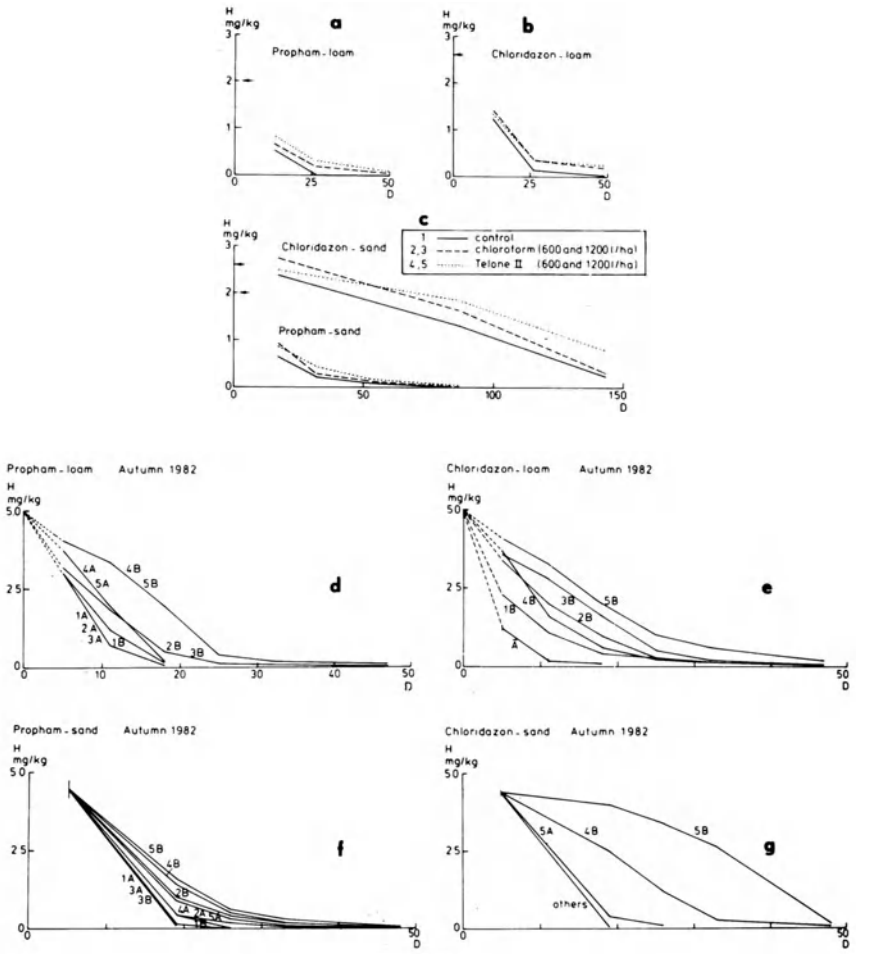

Fig. 2. Degradation of two herbicides in disinfested field plots (a, b, c) and during incubation of samples from these plots (d–g); experiment 3. A = layer 0–10 cm, B = layer 10–20 cm

However, in samples amended with lucerne and then fumigated, propham degraded much slower, and chloridazon required a long lag phase, whereas lucerne alone increased herbicide degradation rates (Fig. 3a–d).

Field fumigation with high dosages of $CHCl_3$ or Telone II in autumn (experiment 3) had little or no effect on herbicide degradation in plots of soil 1 (SL). In plots of soil 2(S) fumigated with Telone II chloridazon disappeared more slowly. Actually, in spring next year chloridazon residues were two to four times higher in the Telone II plots than in the other plots (Fig. 2a–c). In the laboratory, chloridazon and propham disappeared almost at the same rate in all samples from the 0–10 cm

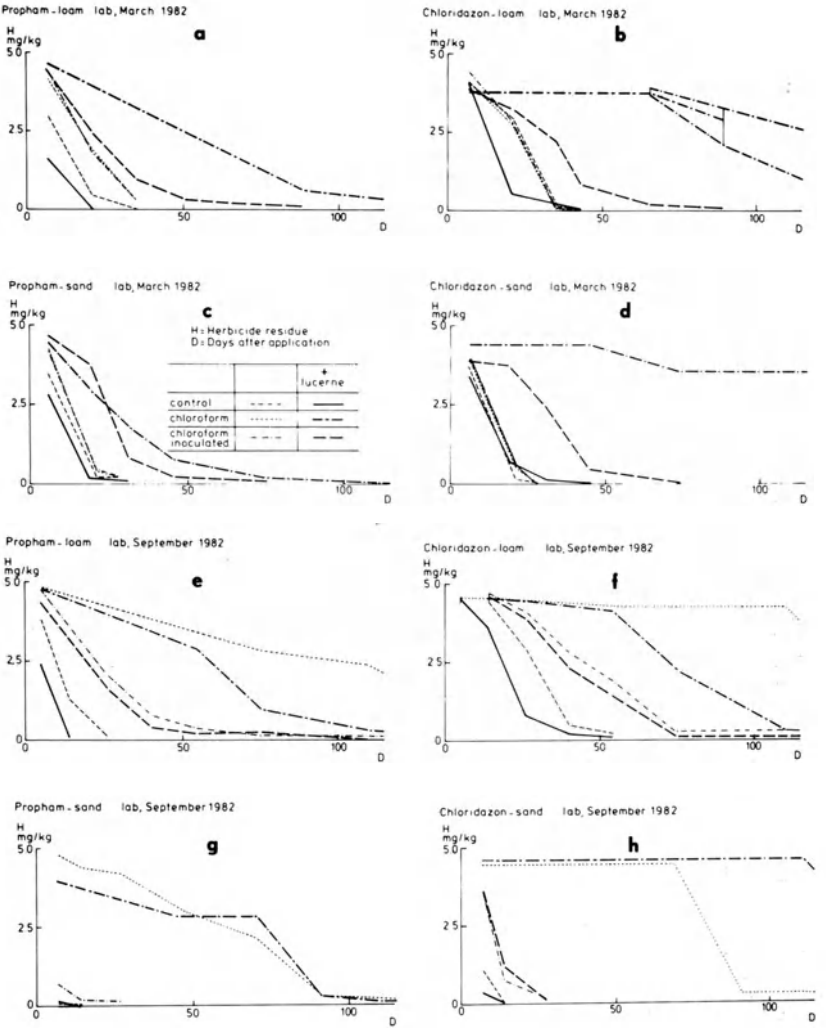

Fig. 3. Degradation of two herbicides in $CHCl_3$ fumigated soil samples with and without lucerne, with and without inoculation with 10% fresh soils; experiment 4

layer, but in samples from the 10–20 cm layer of the $CHCl_3$, and especially Telone II plots decreased herbicide degradation rates were found (Fig. 2d–g). The more rapid disappearance of $CHCl_3$ than of 1,3-dcp may be the main explanation. Even at the highest fumigation dosages the herbicide degradation was complete within 30 to 50 days.

$CHCl_3$ fumigation of soil samples taken in autumn (experiment 4b) greatly inhibited or decreased herbicide degradation, with and without lucerne (Fig. 3e–h). The microflora may already have been more active

Table 3. Effects of soil disinfestation with chloroform and Telone II on biological activity in soil, and number of bacteria and nematodes

Trial (Exp. No.)	Layer*	N-min. (mg/kg) From s o m** 6 wk	From s o m** 12 wk	From lucerne 6 wk	From lucerne 12 wk	Nitrification mg NO_3–N/kg 2 wk	Nitrification mg NO_3–N/kg 6 wk	Urease activity (μg per g/h) 6 wk	Urease activity (μg per g/h) 12 wk	Nematodes (per 200 g)	Bacteria (×10^6/g)	B O D*** (mg/kg) 2 wk
		Soil 1 (S)										
Field autumn (3)												
control	A	15	25	23	41	37	47	7.6	7.7	1700	13	1550
	B	14	20	24	49	39	46	7.9	7.7	2075	15	1450
Cf 600	A	18	29	16	48	29	51	6.8	5.0	500	16	1550
	B	19	26	9	43	28	52	7.0	5.7	625	17	1600
Cf 1200	A	17	24	19	64	35	52	8.3	8.4	475	16	1950
	B	17	26	23	63	33	55	7.6	7.9	600	25	1650
T 600	A	10	17	16	51	5	48	4.9	3.5	0	26	1450
	B	14	21	32	77	0	7	3.8	3.9	0	47	1600
T 1200	A	11	18	16	53	0	24	3.8	2.6	0	40	1400
	B	13	23	32	83	0	3	4.2	2.4	0	60	1400
Laboratory, spring + lucerne (4a)												
control		44	56	33	48	46	74	17.4	15.1	nd	nd	1950
Cf		44	43	3	11	1	0	8.0	8.0	nd	nd	1950
mix 1 : 9		51	68	32	40	3	51	8.0	5.0	nd	nd	2000
Laboratory, spring 4a												
control		14	18	30	50	nd	nd	11.3	11.6	nd	nd	1850
Cf		21	27	5	9	nd	nd	3.3	2.9	nd	nd	1700
mix 1 : 9		22	31	18	36	nd	nd	3.9	4.2	nd	nd	1800
Laboratory, autumn 4b												
control		13	21	29	44	33	45	7.5	7.1	3400	20	1650
Cf		18	25	19	41	0	0	3.0	1.7	0	2	1550
mix 1 : 9		23	31	26	40	2	25	3.1	2.9	175	nd	1800
		Soil 2 (SL)										
Field, autumn (3)												
control	A	13	19	30	42	124	125	6.5	6.5	2400	30	1800

Table 3 (*Continued*) . . .

Trial (Exp. No.)	Layer*	N-min. (mg/kg) From s o m** 6 wk	From s o m** 12 wk	From lucerne 6 wk	From lucerne 12 wk	Nitrification mg NO_3–N/kg 2 wk	Nitrification mg NO_3–N/kg 6 wk	Urease activity (μg per g/h) 6 wk	Urease activity (μg per g/h) 12 wk	Nematodes (per 200 g)	Bacteria (× 10^6/g)	B O D*** (mg/kg) 2 wk
	B	9	12	26	45	123	125	5.3	4.8	1950	13	1700
Cf 600	A	20	33	40	55	96	134	7.4	5.7	600	51	1900
	B	9	12	27	47	34	128	3.8	3.7	575	24	1650
Cf 1200	A	20	28	39	49	80	134	6.6	6.6	400	80	1650
	B	11	18	32	43	15	133	3.7	3.9	500	21	1700
T 600	A	17	28	41	63	25	126	3.9	2.5	0	218	1700
	B	14	22	40	50	8	132	3.3	2.5	0	182	1850
T 1200	A	16	24	29	52	0	126	3.5	2.4	0	141	1950
	B	11	18	34	53	0	40	2.5	2.3	0	191	1600
Laboratory, spring + lucerne (4a)												
control		27	43	18	33	123	130	18.0	14.1	nd	160	2100
Cf		29	37	12	14	0	0	1.4	1.1	nd	1	1800
mix 1 : 9		40	61	23	27	0	94	5.7	5.3	nd	nd	2000
Laboratory, spring 4a												
control		11	19	22	35	93	106	4.3	4.6	nd	10	1500
Cf		14	17	4	11	0	0	0.9	0.7	nd	0.05	1550
mix 1 : 9		16	25	13	24	0	27	2.3	2.2	nd	nd	1600
Laboratory, autumn 4b												
control		15	19	24	44	114	131	7.6	6.3	4200	61	1850
Cf		18	21	1	7	1	1	1.2	0.6	0	0.2	1800
mix 1 : 9		28	35	32	35	3	146	2.9	2.8	300	nd	1750

* A = layer 0–10 cm, B = layer 10–20 cm.
** s o m = soil organic matter.
*** B O D = biological oxygen demand.
nd = not determined.

in these samples due to presence of the crop, and therefore more vulnerable than in spring.

The sandy soil samples were taken from the control plots of experiment 2, and thus had a microflora that was adapted to chloridazon and propham. The very rapid degradation of the herbicides in the unfumigated samples confirms this. This may have influenced the effect of the $CHCl_3$ fumigation. Inoculation with 10% fresh soil rapidly restored herbicide degradation.

Comparison of the results of experiments 3 and 4b indicated that, in the field, part of the soil had escaped fumigation. Other experiments confirm the difficulty of fumigating the top few cm of soil with volatile chemicals. In spring the herbicides will predominantly stay in the top 0–5 cm of the soil, where fumigation has least effect. This explains why fumigation in the field hardly affected herbicide degradation. In winter, the herbicides can move downwards into a layer where fumigation is more effective.

Monitoring for herbicide residues (experiment 5) showed that annually repeated heavy fumigation in autumn did not affect the degradation of herbicides applied in the following year. The crop rotation and its concomitant use of herbicides had a greater effect on herbicide residue levels than fumigation. Four granular nematicides (Mocap, Temik, Vydate and Curaterr) applied in spring were also found to have no effect on herbicide degradation, but the number of different combinations was too small to allow general conclusions for this class of nematicides.

Effects on biological activity

Spring application of $CHCl_3$ in the field (Table 2, exp. 2) did not kill the nematodes or affect microbiological activities significantly. Enrichment with dried ground cow manure and urea tended to increase the number of bacteria and enhanced biological activity without causing greater susceptibility to the fumigant. The rapid disappearance of $CHCl_3$ from soil despite the plastic cover may explain this. The results of soil disinfestation in the laboratory and field (Table 2, exp. 3) are summarized in Table 3.

Soil fumigation in autumn in the field resulted in unchanged or increased N mineralisation: the latter due to decomposition of killed biomass. In both soils, but especially in the sandy soil, N mineralisation after addition of lucerne was stimulated by Telone II, whereas $CHCl_3$ at the highest dose gave this effect only in the sandy soil. Whereas $CHCl_3$ caused a very short retardation of nitrification only in the sandy loam soil, mainly in the 10–20 cm layer, Telone II strongly inhibited

nitrification in both soils, particularly in the 10–20 cm layer and at the highest dosage. In the sandy loam soil, nitrification recovered much faster; after 6 weeks only in the deeper layer at the highest dosage was nitrification still reduced. Near-optimum pH conditions in the sandy loam soil favoured fast recovery of nitrification.

Whereas Telone II reduced the urease activity in both soils in both layers, $CHCl_3$ was only effective in the deeper layer in the sandy loam soil. The more effective elimination of nematodes, but also the greater increase in bacterial numbers, a well-known phenomenon of partial sterilisation, is indicative of the stronger impact of Telone II. However, no differences in O_2 consumption were found. Different behaviour of the soils towards soil fumigation in the field can probably be attributed to differences in physical and chemical qualities and in composition of the microflora.

Soil fumigation with $CHCl_3$ in the laboratory generally resulted in a small increase in N mineralisation. Addition of 11% untreated soil further stimulated N mineralisation. $CHCl_3$ strongly reduced N mineralisation from lucerne, but at most only slightly reduced O_2 consumption. Mixing the sample with 11% untreated soil largely restored mineralisation of lucerne-N. Urease activity was strongly depressed and adding untreated soil did not completely restore it. The absence of nitrification pointed to the elimination of nitrifiers by chloroform; mixing with 11% untreated soil did not restore nitrifying capacity within 6 weeks. In cases checked, nematodes were completely killed in both soils and reduction in bacterial number, measured immediately after fumigation, was also drastic.

Field application of $CHCl_3$, even in large amounts, failed to give as strong an impact as could easily be obtained in the laboratory. Only minor effects were found in autumn mainly in the deeper layer of the sandy loam soil.

The effect of soil fumigation with Telone II at 4 to 8 times the normal dosage on nitrification and urease activity was comparable with the effect of mixing $CHCl_3$-treated soil with 11% untreated soil. The effect on N mineralisation from lucerne was different: in Telone II-treated soil, mineralisation was stimulated; in the mix it was somewhat reduced.

From the laboratory experiments it can be seen that, to achieve a prolonged reduced microbiological activity in soil, an effective kill of microorganisms is necessary. Such a drastic effect in the field is undesirable because it would lead to reduced soil fertility. Fortunately, the risk of such a drastic effect under field conditions appears to be very small.

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